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A

Aspects of Heterophylly in *Morus alba* L.

by

Rosemary J. MacMillan

A dissertation submitted to the Graduate Faculty of Biology in
partial fulfillment of the requirement for the degree of Doctor
of Philosophy, The City University of New York.

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Abstract

Aspects of Heterophylly in Morus alba L.

by

Rosemary MacMillan

Advisor- Professor Dwight T. Kincaid

These experiments were undertaken to test the null hypothesis that there is no difference between lobed and unlobed Morus alba L. leaves beyond the obvious morphological one. Physiological measurements of lobed and unlobed leaves were made of cloned saplings of Morus alba two years in succession. A droughting experiment was added in the second year, as was a mature (dbh 26 cm) tree. Measurements included net photosynthetic rate (P_{net}), transpiration rate, stomatal conductance, water use efficiency (WUE), and leaf water potential. Significant differences were observed. Two-lobed leaves had higher P_{net} production than unlobed leaves.

Five hundred Morus alba leaves on street trees in Manhattan and the Bronx in

New York City, and in Dutchess county (90 miles north of New York City) were measured for leaf chlorophyll content, leaf specific mass (LSM), internodal distance, internodal circumference, leaf area, petiole length, blade length, blade width, perimeter, dissection index, and leaf complexity. Significant differences were found. Lobed sun and shade leaves had less blade area than unlobed leaves, more chlorophyll, and were more proximally situated on a shoot.

A census was made of every leaf of 18 saplings and of selected shoots from other trees to investigate patterns in lobing. A highly significant correlation was found between shoot side and lobe(s) side. Lobed leaves on the left side of the shoot were far more likely to have lobes on their left side and vice versa. It was established that overall on a plant, leaves are bilaterally symmetrical in terms of numbers of lobes.

Stomatal density and anatomy were measured for lobed and unlobed leaves in sun and in shade, and with added nitrogen and with severely reduced nitrogen nutrition. More and larger stomates were observed in unlobed leaves in sun plants and in nitrogen supplied plants; more and larger stomates were found in lobed leaves in shade plants and in nitrogen deprived plants.

Overall, it was shown a lobed Morus alba leaf is a different physiological, morphological (beyond lobing), and anatomical organ than a unlobed Morus alba leaf.

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One of the main reasons I took this difficult road was to provide a role model for my daughter, Sylvie Leftwick. I hope it will serve as well for my soon to arrive granddaughter.

INTRODUCTION

In Japan, China, India, and to a lesser extent in France and Italy, Morus alba L. is cultivated as the only feeding source for Bombyx mori, the silkworm. Dozens of varieties of Morus alba are grown at the Silkworm Institute in Lyons, France always with an eye to the most prolific leaf production for different ecological environments (pers. observation). In New York City (Latitude: 40°39'N) Morus alba is culled regularly as a weed tree from any parks or other public land where it manages to grow in sidewalk cracks, alleys, fence lines and other equally inhospitable sites. These two extremes of attention point to both the importance and the vigor of the species. On the one hand it can be grown in an unending variety of form and is a vital component for the silk industry; on the other it is successful in the harshest urban ecological environments. Its phenotypic plasticity may be a reason for its success in the latter (Gray 1990).

A perfect example of this is the single well-established specimen of Morus alba (dbh 26 cm) growing above a rise of the approach to the Cross Bronx Expressway in Manhattan and continuously bathed in auto exhaust fumes. The only indication of a response to this harshest of environments is the almost total absence of unlobed leaves on the tree and the extreme degree of lobing of its leaves. This tree exemplifies the established fact that Morus produces lobed leaves in response to stress (Gray and Call 1994). What is not addressed by Gray and Call, or in the general literature, or at the Silkworm Institute, is whether or how a lobed leaf differs from an unlobed leaf in its response to the environment and in its relationship to the plant in general.

To be sure, there are numerous studies on heterophylly in general beginning with Goebel in 1900. The phenomenon can be observed in many families and has been studied from many angles. Heterophylly can be a predictable phase of a species' growth and resulting from leaf ontogeny, shoot growth cycle, phase change, or dimorphism, or it can be an irregular, although often patterned, occurrence ascribable to various ecological influences including microclimate and predation (Critchfield 1970; Feldman and Cutter 1970; Jones 1995; Klein et al. 1992; Smith 1966; Sparks and Postlethwait 1967; Talbert & Holch 1957; Thomas 2000).

None of these works address the particular case of Morus alba which exhibits hererophylly to an extreme degree. But it is this very extremeness of lobing that makes the species a good subject for studying heterophylly. Environmental factors can almost be ruled out since lobed and unlobed leaves are habitually found on the same shoot only an internode away. And, though even an internode's length involves differences in microclimate, no study of two leaves or more of any species would be possible without overriding this fine point of difference. With this in mind, the experiments described in the following pages were undertaken with a view to probing Morus alba leaves for differences in physiology, anatomy, and ecology, and morphology beyond the fact of lobing.

Background

Botanical classification of mulberry

Morus alba L. is in the genus Morus, in the family Moraceae, in the order Urticales, in the subclass Hamamelidae. With 40 genera and 1,000 species, the family has global representation (Cronquist 1981). The genus Morus is represented in Eurasia and in North America. Other well-known members of Moraceae include Ficus (the banyan tree and the fig tree), Maclura (the osage orange tree) and Artocarpus (the breadfruit tree). All have in common milky juice.

Morphology

Morus alba is a tree that grows to 20 meters (Bailey 1963) although it often is maintained as a shrub when it is used as a food source for the silkworm, Bombix mori. Morus alba is monoecious or dioecious, has cylindrical, catkin-like spikes of staminate flowers, a 4-parted perianth, and a 2-parted style. The fruit is a berry-like syncarp that ranges in color from white to nearly black and is very delicate which may explain why it is not harvested commercially.

The leaves of Morus alba are simple and toothed and range in form from unlobed to with as many as 12 lobes. I have defined a lobe for this study as any easily visible untoothed, curved break in the otherwise continuously toothed perimeter of a leaf. Morus alba leaves are palmately veined. Their arrangement is alternate with variable phyllotaxy (Esau 1960 , Guedes 1979). I have observed instances of both 1/3 and 2/6 phyllotaxy. The leaves are glabrous except for tufts of hairs found on the abaxial side at the junctions of the major veins. This characteristic is a major distinguishing feature between Morus alba and the native North American tree Morus rubra L., the latter having hairs more evenly distributed over the leaf (Dr. M. Nee, pers. comm.) Winter identification of the two species is possible since M. alba buds are 2 to 4 mm in length and appressed while M. rubra has buds between 5 and 7 mm long and somewhat divergent (Braun 1961, Dirr 1990). The bark of the youngest shoots and of exposed roots is a distinct pale orange which is helpful as a winter identification key.

History

White mulberry, Morus alba, is one of 12 species in the genus Morus (Bailey 1963). Of these, only two, the red mulberry, Morus rubra,. and the Texas mulberry, Morus microphylla Buckl., are native to the United States. White mulberry and the black mulberry, Morus nigra L, are Asian (Elias 1980). Red and white mulberry each have 28 chromosomes and hybrids have been recognized (Williams 1981).

There are New York Botanical Garden herbarium sheets locating Morus alba all along the east coast from Massachusetts down to northwestern Florida where it is common in disturbed sites (Nelson, 1994) and as far west as Utah where it is represented in several locations, all at elevations of 1,500 meters or more. It is rare in most of the western U.S except for California (Duncan 1988). Morus rubra is found along the east coast, too, although not nearly as often as M. alba in New York City and its environs. It is generally accepted that the presence of Morus alba in North America is due to several aborted attempts to establish a silkworm industry in America in the 1600's and 1800's, and to the propensity of Mediterranean immigrants in the early 20th century to bring cuttings of this favorite tree with them.

Morus alba takes it place among economically important plants as the favored food of the monophagous silkworm, Bombyx mori (Mahmood 1987) and has been in cultivation in Asia for thousands of years. The attraction of the leaf to the silkworm is apparently its extremely high concentrations of sucrose (Yazawa 1983). Morus alba leaves are also of pharmacological interest for their use in powdered form as a tea drunk in Japan which is thought to alleviate high blood pressure (Shimizu 1992). It is reported as well that unripe fruit and raw shoots of Morus alba contain an hallucinogen (Williams 1981). Chinese herbals for the last two thousand years list numerous uses for Morus alba shoots, leaves and root bark (Ling 1995). No mention is made by any of these authors as to any sorting of leaves by lobing.

Morus alba has the dubious distinction of being on the New York City Parks Department's most unwanted list. Where it exists as a single, mature specimen it seems to find acceptance, but my observations in the last few years have shown that regular sweeps are made of volunteer mulberry saplings on parkland. It is a common backyard tree and very successful at seeding itself in empty lots.

CONTENTS

Chapter 1 - Physiological data for lobed and unlobed leaves.

SECTION 1 1998 data

SECTION 2 1999 data

SECTION 3 Droughting experiment

SECTION 4 Data from mature specimen

SECTION 5 Water potential experiment

Chapter 2 - Stomatal characteristics

Chapter 3 – Leaf census

Plant material and location
Description of measurements
Results

List of tables

List of Figures

CHAPTER I

PHYSIOLOGICAL DATA FOR LOBED AND UNLOBED LEAVES ON MORUS ALBA SAPLINGS AND ON A MATURE SPECIMEN

SUMMARY

Midday gas exchange responses including P_{net}, transpiration, and stomatal resistance were measured for lobed and unlobed leaves on inground saplings established in a plantation in Columbia County in New York State 100 miles north of New York City and on a mature tree in the Bronx. PAR (photosynthetically active radiation) and leaf temperature were also measured. Data were taken over the course of two summers. A droughting experiment was conducted with potted plants in this same plantation. A Scholander chamber was used to determine leaf water potential. Plants were classified as being in sun or in shade. Sun plants were in full sun. Shade plants were growing under 60% shade cloth cover.

The1998 measurements categorized leaves as being unlobed, few-lobed (1,2,3) or many-lobed (4+). Few-lobed leaves in sun had higher P_{net} (net photosynthesis) and transpiration means than unlobed leaves; in shade P_{net} was higher as well for few-lobed but without statistical significance while transpiration was significantly higher. Many-

lobed sun leaves were almost identical to unlobed leaves in P_{net} readings; many-lobed shade leaves had significantly lower P_{net} than unlobed leaves. In both sun and shade, many-lobed leaves had lower transpiration rates and higher stomatal resistance rates.

Data recorded in 1999 disclosed no differences between lobed and unlobed leaves in P_{net}, transpiration or stomatal resistance except for the two-lobed shade leaf group that had significantly higher P_{net} over unlobed leaves.

In both years PAR and leaf temperature were influential as covariance effects in ANCOVA.

The droughting experiment was conducted in the shade enclosure using potted plants. In the control plant, lobed leaves had a significantly higher transpiration rate than unlobed leaves. In the droughted plant, the P_{net} and transpiration readings taken after the longest period of complete drought (nine days) showed lobed leaves to be significantly more productive than unlobed leaves.

Readings taken on leaves on the mature Morus alba specimen indicate statistically significantly higher P_{net} activity for lobed leaves versus unlobed leaves in the sun. Transpiration rates were similarly higher. With PAR and leaf temperature included as effects, the results for P_{net} and transpiration were just short of significance but the effect sizes were very strong. Correlation between incident PAR and P_{net}, transpiration, and stomatal resistance was always stronger for two-lobed leaves vs. unlobed leaves in sun which could be evidence that lobed leaves are more tightly coupled to the microclimate.

Leaf water potential was measured for the potted plants that had been used in the droughting experiment. It was significantly higher for lobed leaves in the control plant; for the plant that had been droughted, there was no difference in leaf water potential of lobed and unlobed leaves. For the inground plant in full sun, the unlobed leaf was less stressed; the inground shade plant showed no difference in leaf water potential between lobed and unlobed leaves.

Introduction

Mature, well-established *Morus alba* trees in New York City have mostly unlobed leaves. Nonetheless, even these trees often display lobed leaves mixed in an apparently random manner on the same shoot with unlobed leaves. On saplings, suckers, coppiced trees and other white mulberries in less than ideal growing conditions on the other hand, the majority of leaves are lobed. There seems to be general agreement among plant scientists that stress in one form or another triggers lobing behavior in numerous genera. Gray and Call (1994) demonstrated that *Morus rubra*, a close relative of *M. alba* also exhibited lobing behavior as a response to stress in the form of pruning or simulated herbivory by producing a greater number of lobed leaves. Hanson (1917) found that the most exposed leaves of *Acer saccarum* were more deeply lobed. Buisson and Lee (1993 connected dramatically reduced lobing in papaya and the quality of R:FR light under filtered shade. Jones (1995) looked at the effect of shade on lobing in *Cucurbita argyrosperma* Pangalo and concluded that the less-lobed shade leaves were a result of prolonged juvenility as well as a plastic response to shade on the part of the leaf. Smith (1966) saw that the late season leaves of *Liquidambar styraciflua* L. were more lobed. He ascribed this to the plant's insuring against late summer drought by producing lobed leaves with no cells as far removed from the main vein of the leaf as would be the case in unlobed leaves.

I have observed typical M. alba saplings, which had previously exhibited "normal" lobed/unlobed behavior, respond to extreme winter herbivory by squirrels by producing only lobed leaves the following spring. If stress is the signal for lobing, why is a M. alba beneficial for the plant? In the following experiments the physiological behavior of lobed and unlobed leaves in sun and shade and in a drought situation was examined. P_{net}, transpiration, and stomatal resistance were measured as were their interrelationships and the correlation of these responses to incident PAR.

SECTION I

Materials and Methods

The eight plants used in this first experiment were three-year old Morus alba saplings originating from cuttings taken from trees in Manhattan, the Bronx, and Columbia county in New York State, grown in identical glasshouse conditions and transferred a full year before the measurements were made to a plantation situation in Columbia county. Ranging in height from 90 to 150 cm, all of the saplings were planted in-ground , some exposed to full sun and some placed under a permanent canopy of 60% shade cloth (Green-Tek, Inc. aluminet). The overall sample size is 288 measurements made of lobed and unlobed M. alba leaves.

The data were recorded in late August of 1998 from mid-morning to mid-afternoon. Each leaf was measured three times in rapid succession; 96 leaves in all were actually measured. These are not considered repeated measures because they were taken over the course of one minute. They are, however, considered as separate measurements because the mean for each measurement is different and reflects a unique instant in time with slightly different microclimatic states.

To collect the data an infrared gas analyzer-based photosynthetic system (Model LI 6200, LI-COR Inc., Lincoln, Nebraska) was used. Gas exchange variables measured included:

Variable	Units
Net photosynthesis (P_{net})	(μmol m^{-2} s^{-1})
Transpiration	(mol m^{-2} s^{-1})
Stomatal resistance	(s cm^{-1})
Photosynthetically active radiation (PAR)	(μmol m^{-2} s^{-1})
Leaf temperature	(C°)
Water use efficiency (WUE)	(ratio)

With the information generated by the gas analyzer, water use efficiency was computed as the ratio of P_{net} to transpiration rate. The area of each leaf measured was four square centimeters and care was taken that it not include any major veins.

An equal number of lobed and unlobed leaves were measured. Leaves were chosen on the basis of accessibility to the gas exchange chamber. The youngest leaf was generally avoided even though it may have been fully matured since experience has shown that, although similar in area to its fellows, this leaf is often thinner to the touch and may therefore be different in physiological aspects as well. As a check that the LICOR 6200 was in working order and being operated correctly, an ANOVA was performed on the 288 measurements using PAR (photosynthetically active radiation) as the variable response and Site as a nominal category. Mean PAR in sun 1595 mol per $m^{-2}s^{-1}$ +/-SD 352, and 540 mol per $m^{-2}s^{-1}$ +/-SD 171 in shade. The expected highly statistically significant result was an F statistic of 700 and a P value of <0.0001.

For statistical analysis the programs JMP (ver. 3.2.1) and Statview (ver. 5.0.1) were used on Macintosh computers. Analyses in this section generated from JMP and Statview computer files "96 lvs 98."

Results

The results were examined on several levels. Initially, the leaves were categorized as sun and shade, and as"all-lobed" and "unlobed." The lobed leaves were assigned to two separate subcategories: "few" leaves with one, two, and three-lobes, and "many" leaves with four or more lobes. Descriptive statistics were generated for all four groups in the areas of P_{net}, transpiration, stomatal resistance, and WUE (Table 1-I). A second level of

Table 1-I. Descriptive statistics for N=288 observations (96 leaves, 3 readings per leaf). Mean and standard deviation for lobed and unlobed leaves, and for lobed leaves decomposed by 1,2,3-lobed, and 4+lobed groups; further categorization by breakdown of site, all measured for photosynthesis (μmoles per m^2s^1), transpiration (mmoles per m^2s^1), stomatal resistance (s cm^1), and WUE (water use efficiency).

N	Lobe Category	Site	Photosynthesis	Transpiration	Stomatal Resistance	Water Use Efficiency
66	Unlobed	Sun & shade	10.277 (2.954)	2.752 (0.239)	3.522 (0.310)	4.217 (1.391)
222	Lobed	Sun & shade	11.889 (4.601)	3.296 (0.207)	3.143 (2.365)	4.658 (2.450)
120	1,2,3-lobed	Sun & shade	13.690 (4.876)	4.186 (2.058)	2.321 (1.923)	3.675 (1.191)
102	4+lobed	Sun & shade	9.771 (3.149)	2.248 (1.550)	4.110 (2.476)	5.814 (2.994)
51	Unlobed	Sun	9.176 (2.243)	2.178 (0.788)	4.235 (0.975)	4.529 (1.385)
144	Lobed	Sun	10.618 (4.205)	2.262 (1.300)	4.362 (2.056)	5.583 (2.527)
72	1,2,3-lobed	Sun	12.181 (4.543)	3.039 (1.292)	3.404 (1.770)	4.249 (1.105)
72	4+lobed	Sun	9.056 (3.164)	1.486 (0.715)	5.321 (1.874)	6.917 (2.834)
15	Unlobed	Shade	14.017 (1.775)	4.703 (1.391)	1.106 (0.575)	3.155 (.750)
78	Lobed	Shade	14.240 (4.395)	5.204 (1.887)	0.891 (0.532)	2.949 (.900)
48	1,2,3-lobed	Shade	15.953 (4.506)	5.910 (1.778)	0.696 (0.376)	2.812 (.693)
30	4+lobed	Shade	11.488 (2.387)	4.079 (1.486	1.202 (0.599)	3.167 (1.136)

investigation examined the leaves by lobe in sun and shade together. On a third level all sun and all shade leaves, irrespective of lobing condition were observed (Table 1- II).

All-lobed/unlobed leaves in sun

A statistically significant difference was seen between all-lobed versus unlobed means in a one-way ANOVA of P_{net} (F= 5.43, v1,v 2= 1,193, P= 0.02). The mean for lobed leaves was 10.6; for unlobed 9.2. The Rsq is only 0.03. When PAR and leaf temperature are entered as covariates in ANCOVA in an attempt to strengthen the Rsq, neither explains a significant proportion of the sum of squares (P = 0.09 and 0.14, respectively).

The one-way ANOVA for transpiration showed a higher mean for lobed leaves but not with significance. Adjusted means in one-way ANCOVA with PAR and leaf temperature entered as multiple covariates generated an F of 29.42 and a P < 0.0001. All effects in the model were significant and the Rsq was 0.32. There was a significant difference in leaf temperature between the two types of leaf (F= 43, P <0.0001) with the unlobed leaf mean (28.1) being higher than the lobed leaf mean (26.3) which corresponds to the lobed leaves having a numerically higher average rate of transpiration. A Manova (Bray 1985, Manly 1986) with a low F statistic (3.13) but a P of 0.03 showed P_{net} and stomatal resistance to be equally important in separating out the multivariate means between lobed and unlobed leaves (Fig.1-1).

Table 1- II. Descriptive statistics and one-way ANOVA for N=288 observations (96 leaves, 3 readings per leaf). Mean and standard deviation for photosynthesis (μm m^2s^1), transpiration (m m^2s^1), stomatal resistance (s cm^1), leaf temperature (C^o), PAR (μm m^2s^1) and WUE (μm m^2s^1)/(m m^2s^1) for <u>Morus</u> <u>alba</u> sun and shade leaves.

	SUN	N=195	SHADE	N=93	One-way ANOVA	
	Mean	Std. Dev.	Mean	Std. Dev.	F	P
Leaf temp.	26.765	3.976	25.175	3.029	12	0.0007
Photo	10.241	3.838	14.201	4.081	64	<0.0001
RS	4.329	1.834	.924	.541	308	<0.0001
Transpir	2.240	1.186	5.123	1.810	261	<0.0001
PAR	1619.075	332.513	539.625	171.016	869	<0.0001
WUE	5.307	2.327	2.982	.877	87	<0.0001

All-lobed/ unlobed leaves in shade

There were no statistically significant differences on any level here (Fig.1-1).

Few-lobed/unlobed leaves in sun

Few-lobed leaves photosynthesize and transpire significantly more than do unlobed leaves, and have lower stomatal resistance. There is no strong correlation between these responses and the amount of incident PAR (photosynthetically active radiation) on the part of either type of leaf (Table 1- III). A grand mean generated in a Manova shows photosynthesis as the major contributing factor to the significant difference between few-lobed/unlobed leaf physiological response, followed closely by stomatal resistance (Fig.1-2). WUE, a leaf score derived by dividing P_{net} by the transpiration rate, which may be deemed to assign value to the leaf, is not significantly different between unlobed leaves and lobed leaves in sun (Table 1- IV).

Few-lobed/unlobed leaves in shade

There was no statistically significant difference between few-lobed and unlobed leaves in P_{net} ,although the few-lobed mean was higher (Tables 1- I,1- IV). In transpiration few-lobed leaves have significantly higher means than do unlobed leaves; in stomatal resistance the few-lobed leaf mean is significantly lower. The grand mean in a Manova does show significant difference between few-lobed leaves and unlobed leaves but it is the stomatal resistance effect that is by far the most important contribution to the result

Table 1- III. Photosynthesis, Transpiration, & Stomatal Resistance correlation to increasing PAR by few-lobed, unlobed, and many-lobed *Morus alba* leaves in sun and in shade.

N	Leaf Type	Site	Photosynthesis		Transpiration		Stomatal Resistance	
			Correlation	P value	Correlation	P value	Correlation	P value
72	Few-lobed	Sun	0.076	0.52	0.117	0.33	0.202	0.09
51	Unlobed	Sun	0.134	0.35	0.196	0.17	-0.038	0.79
72	Many-lobed	Sun	-0.041	0.73	0.439	0.0001	0.095	0.43
48	Few-lobed	Shade	0.428	0.002	0.529	0.0001	-0.432	0.002
15	Unlobed	Shade	0.077	0.78	-0.202	0.47	0.341	0.21
30	Many-lobed	Shade	0.062	0.74	0.318	0.09	-0.144	0.45

Table 1-IV. ANOVA results. Twenty four one-way ANOVAS. Power is calculated at 0.05. N shows both group numbers.

Source of variation	DF	SS	F	P	Rsq	Power	Minimum sample size	N
Photosynthesis								
None vs. few (sun)	1	269.5	19.0	<0.0001	0.14	0.99	27	51/72
None vs. many(sun)	1	.4	.06	0.8158	0.00	0.06	8,672	51/72
Few vs. many (sun)	1	351.6	22.94	<0.0001	0.14	0.99	26	72/72
None vs. few (sh)	1	42.9	2.62	0.1108	0.04	0.36	94	15/48
None vs. many (sh)	1	63.9	13.13	<0.0008	0.23	0.94	15	15/30
Few vs. many (sh)	1	367.9	24.97	<0.0001	0.25	1.0	14	48/30
Stomatal Resistance								
None vs. few (sun)	1	20.7	9.26	0.0029	0.07	0.85	53	51/72
None vs. many(sun)	1	35.2	14.33	0.0002	0.11	0.96	35	51/72
Few vs. many (sun)	1	132.4	39.82	0.0001	0.22	1.0	16	72/72
None vs. few (sh)	1	1.8	9.85	0.0026	0.14	0.87	27	15/48
None vs. many (sh)	1	.1	.32	0.5723	0.01	0.09	536	15/30
Few vs. many (sh)	1	4.7	21.02	<0.0001	0.25	0.99	16	48/30
Transpiration								
None vs. few (sun)	1	22.2	17.92	0.0001	0.13	0.99	28	51/72
None vs. many(sun)	1	14.3	25.66	<0.0001	0.18	0.99	20	51/72
Few vs. many (sun)	1	86.9	79.69	<0.0001	0.36	1.0	9	72/72
None vs. few (sh)	1	16.6	5.85	0.0186	0.09	0.66	43	15/48
None vs. many (sh)	1	3.9	1.90	0.1749	0.04	0.27	93	15/30
Few vs. many (sh)	1	61.7	22.03	<0.0001	0.23	1.0	16	48/30
Water Use Efficiency								
None vs. few (sun)	1	2.3	1.6	0.2162	0.01	0.23	308	51/72
None vs. many(sun)	1	170.2	30.9	0.0001	0.20	0.99	17	51/72
Few vs. many (sun)	1	256.1	55.34	0.0001	0.28	1.0	12	72/72
None vs. few (sh)	1	1.3	2.7	0.1057	0.04	0.37	92	15/48
None vs. many (sh)	1	.001	.001	0.9707	0.00	0.05	126,755	15/30
Few vs. many (sh)	1	2.3	2.94	0.0900	0.04	0.39	104	48/30

(Fig. 1- 2). The unlobed shade leaf does not respond to greater incident PAR with any increase in P_{net} whereas the few-lobed shade leaf's P_{net} output is directly and positively correlated to increasing light (Table 1- III, Fig. 1- 4). A stronger positive relationship exists between incident PAR and transpiration for few-lobed leaves (Fig.1- 3). The transpiration rate of unlobed leaves decreases with increasing PAR (Fig.1- 4) but not to the point of statistical significance. The stomatal resistance of the lobed leaf shows a significant negative correlation to increased PAR (which increases the heat load). The unlobed leaf shows a positive but nonsignificant response to increased PAR (Table 1- III). Water use efficiency is not significantly different between the two types of leaf.

Many-lobed/unlobed leaves in sun

The P_{net} means for many-lobed leaves and unlobed leaves were almost identical; neither leaf type shows any correlation between photosynthesis and PAR. The transpiration rate for unlobed leaves was significantly higher than that of lobed leaves though the correlation between transpiration and quantum was not significant for unlobed leaves but was highly significant for lobed leaves (Fig. 1- 4). The stomatal resistance of unlobed leaves was significantly lower than that of lobed leaves. The difference in transpiration and lack of difference in P_{net} behavior resulted in a significantly higher WUE rating for many-lobed sun leaves. Overall though, a Manova generated in JMP which shows significance ($F=11$, $P < 0.0001$) indicates the difference in stomatal resistance between the two types of leaf as having the greatest effect (Fig.1- 3).

Many-lobed/unlobed leaves in shade

The P_{net} mean for unlobed leaves was significantly higher than that for many-lobed leaves. Neither leaf type showed any correlation between photosynthesis and PAR. Transpiration and stomatal resistance means between the two types of leaf were not significantly different, nor were the WUE ratings. However, the responses of the two leaf types to increasing PAR *vis a vis* transpiration were opposite (Fig.1- 4). Transpiration increased with increasing PAR for the lobed leaf. Transpiration declined to almost the same degree to increasing PAR for the unlobed leaf. In neither case was the correlation significant (Table 1- III).

Discussion

In examining the entire set of lobed leaves versus unlobed leaves in sun, only P_{net} and leaf temperature were statistically significantly different with the lobed leaf having the higher P_{net} result and the unlobed leaf the higher temperature. This P_{net} result explained only 3% of the variability between lobed and unlobed leaves. The leaf temperature difference explained only 4% of the variability. In shade, no statistical significance was found. It was necessary to break down the data into subsets based on the amount of lobing present in the leaf in order to pick up greater differences. When this was done, a significant difference of means in behavior responses could be seen between few-lobed leaves and unlobed leaves and an even more pronounced difference in behavior between few-lobed leaves and many-lobed leaves (Fig. 1- 5,1- 6). Based on the results of this experiment no conclusion can be made regarding the physiological behavior of lobed and unlobed M. alba leaves. However, some specific findings are significant. In sun, the few-lobed leaf produces more carbon (on a per mm^2 basis) for the plant than the unlobed leaf. It also transpires more per unit area than the unlobed leaf. Since the WUE rating shows no significant difference between the two types of leaf, it could be interpreted that the few-lobed leaf is contributing more carbon to the plant than the unlobed leaf. That they respond differently to environment is clear. With significantly higher leaf temperature and greater stomatal resistance, both characteristics associated with lower transpiration, the unlobed sun leaf seems to be programmed to shut down much more quickly than the few-lobed sun leaf. This agrees with Givnish's (1979) definition of a broader leaf (read:unlobed) with deep boundary layers tending to impede heat loss, but Givnish also reasoned that higher leaf temperature should increase the rate of transpiration, a finding contrary to that of the experiment under discussion here. The higher leaf temperature reported here for unlobed leaves is similar to Vogel's (1968) finding that the more deeply lobed sun leaves of Quercus alba L. were cooler than shade leaves from the same tree. In any event, whether a lobed or unlobed leaf is an advantage to the tree might depend on the amount of carbon allocation necessary to have produced the leaf initially. A different experiment discussed in Chapter 3 of this paper showed there to be no significant difference in the LSM of lobed and unlobed M. alba leaves. The experiment did not address the proportion of vascular tissues supporting either type of leaf. Dial

(1997) weighed the primary and secondary veins of Passiflora and found that large (7,000 mm^2 +) two-lobed leaves carry less vein mass than large unlobed leaves. Kincaid et al. (1998) found that leaf surface area in Pourouma tomentosa was significantly greater in lobed leaves vs. unlobed leaves though leaf specific mass (LSM) was not. The findings of these experiments point to there being a greater amount of lamina surface in a lobed leaf without the added "expense" of more vascular tissue.

The few-lobed leaf in the shade, with a significantly higher transpiration rate and only a slight P_{net} advantage over an unlobed leaf, also shows no significant difference in WUE compared with an unlobed shade leaf. This few-lobed leaf has a significantly lower stomatal resistance than the unlobed leaf (Table 1- II). What is significant is that the few-lobed shade leaf's gas exchange responses are all related to changes in the incident PAR on the leaf (Table1- IV). The unlobed shade leaf does not respond to any noticeable extent to PAR.

Many-lobed sun leaves are significantly cooler than unlobed leaves (mean of 28.1 C° vs. 25) and shut down (stomatal closure) much more quickly. Sun leaves also have a higher WUE score because of their significantly lower transpiration and higher stomatal resistance rates (Table 1 - I). In shade, the results are similar with many-lobed leaves being cooler, though not significantly, and having lower transpiration and higher stomatal resistance rates than unlobed shade leaves. But then an important difference registers – the P_{net} for the unlobed shade leaf is significantly higher than that of the many-lobed. Considering all three groups – unlobed, few-lobed, and many-lobed, it is seen that the physiological responses of many-lobed leaves and unlobed leaves are quite similar. (Figs. 1-5,1-6).

Few versus many

In both sun and shade few-lobed leaves and many-lobed leaves show highly statistically significant differences in behavior *vis a vis* each other in P_{net}, transpiration and stomatal resistance. . Few-lobed leaves have greater P_{net} than many-lobed leaves. They transpire more, and their stomatal resistance is lower. This result may be related to the significantly different temperatures of these two leaf types in both sun and shade (Fig.1-7). A Manova comparing the three responses together for sun leaves registered transpiration as having the greatest effect on the significant grand mean. For shade leaves, P_{net} and stomatal resistance were the major contributing effects of the significant grand mean. The consistency of difference in behavior between few-lobed leaves and many-lobed leaves is greater than that of either unlobed and few-lobed or unlobed and many-lobed comparisons.

Sun/Shade

Data generated to examine differences between lobed and unlobed leaves also provided information on the behavior of leaves in sun and in shade. A counterintuitive result was the finding that shade leaves, though significantly cooler than sun leaves and receiving only a third as much incident PAR, still photosynthesize more than sun leaves. This advantage is somewhat tempered by the finding that because shade leaves transpire more as well and because of their extremely low stomatal resistance, the WUE score for sun leaves is significantly higher than that for shade leaves (Table 1 –II).

SECTION 2

Materials and Methods

Plants used in this experiment were four-year old Morus alba saplings from the same lot as the 1998 experiment. The data were recorded from the end of May 1999 through mid-September of 1999. It consists of 576 rows representing a total of 292 leaves measured. The procedure followed was the same as in Section One Material and Methods.

Analyses in this section were generated from computer files "MJJAS no 12,13 576 rows" and "With PAR and LT."

Results.

As with the 1998 data, the results were examined on two levels: all-lobed leaves vs. unlobed leaves, and two-lobed leaves vs. unlobed leaves.

Sun Leaves

Neither the all-lobed/unlobed group nor the two-lobed/unlobed group in sun showed any significant difference in P_{net}, transpiration or stomatal resistance examined in one-way ANOVA. There was a significant difference between the all-lobed group and the unlobed group in nodal position score (F= 20, v1, v2= 1,288, P <0. 0001,Rsq = 0.07). The mean for unlobed leaves was 0.769 and for lobed leaves 0.675. This same pattern held for the two-lobed vs. unlobed group.

For the two-lobed vs. unlobed leaves comparison ANCOVA for P_{net} including PAR and leaf temperature as covariates was not significant. For transpiration the overall ANCOVA had a P of < 0.0001 with PAR (P<0.0001) and leaf temperature (P=0.0005) accounting entirely for the significance. For stomatal resistance, the overall ANCOVA had a P value of <0.0001; the only significant effect was PAR (P<0.0001).

Shade Leaves

Neither the all-lobed/unlobed group nor the two-lobed/unlobed group in shade showed a significant difference in transpiration or stomatal resistance examined in one-way ANOVA. For P_{net}, the one-way ANOVA for the two-lobed/unlobed group was significant (F = 9.1, v1,v2 = 1,172, P = 0.003, Rsq = 0.05). The mean for the two-lobed leaves was 13.66; for the unlobed leaves it was 12. The nodal position score for all-lobed vs. unlobed leaves was not significant; for the two-lobed vs. unlobed group there was significance (F = 10.9, v1,v2 = 1,165, P = 0.001, Rsq = 0.06). The unlobed mean was 0.722; the two-lobed mean was 0.628.

An ANCOVA that examined P_{net} in the all-lobed/unlobed group with PAR and leaf temperature as effects was significant (P = 0.005) but the only effect in the test that was significant was PAR. The two-lobed/unlobed P_{net} ANCOVA had an overall P of <0.0001 with the significance coming from the lobing effect (P = 0.003) and the PAR effect (P = 0.001); leaf temperature was not significantly different. In transpiration, the overall significance of the ANCOVA for all-lobed vs. unlobed leaves came from PAR and leaf temperature; in the two-lobed vs. unlobed test, PAR was the only significant effect. In stomatal resistance there was a significant ANCOVA for all-lobed leaves vs. unlobed leaves with PAR being the only significant effect; in the two-lobed vs. unlobed group, it was PAR that contributed to the overall significance of the ANCOVA. The unlobed shade leaf shows slight correlation between P_{net} and incident PAR; for the two-lobed shade leaf the correlation is strong (Fig.1- 8).

Discussion

The 1999 data set was almost twice as large as the 1998 data set and the observations were made from May through September. Nor are the grouping categories of the leaves identical. Lobed leaves in 1999 were examined on two levels: all-lobed or two-lobed. In 1998 they were categorized as being “few-lobed” or “many-lobed”. The “few-lobed” category of 1998 corresponds to the “all-lobed” category of 1999 for sun leaves; for shade leaves the 1999 ‘all-lobed” category is more comprehensive than the1998 “few-lobed” category. A direct comparison of the two years is not the intent of this paper.

As with the 1998 results, the overall lack of significant difference between lobed and unlobed leaves for the 1999 observations, excepting only that of two-lobed shade leaves, does not allow a simple, definitive statement. In 1999, the two-lobed shade leaf produced significantly more carbon per unit area than did the unlobed shade leaf without a concomitant significance in transpiration rate. Moreover, it did this from a less advantageous position (more proximal) on the shoot. If there is a thread that runs through both years, it might be the greater responsiveness of lobed shade leaves to incident PAR in contrast to unlobed leaves.

SECTION 3

Material and Methods

This droughting experiment was conducted on two in-ground four-year old Morus alba plants representing different genets grown from cuttings taken from New York City street trees. Both plants were situated under 60% shadecloth cover. Both plants were potted and left in the shade enclosure. The plant to be droughted was encased in vinyl to prevent water absorption through the bottom of the pot. The vinyl was brought up to cover the top surface of the pot and weighed down by stones. This plant received no water at all for nine days while the control plant was watered freely throughout the experiment period. Gas analysis readings using a LI-COR 6200 were begun after nine days of complete drought and continued for three weeks during which time the plant being droughted was allotted 330ml of H_2O after the ninth day and 660 ml after the 16th day.

Analyses were generated from computer files “Droughted exp. 12/13, 216 rows” and “Drgt exp. 12/13, 216 rows OK.”

Results

Descriptive statistics were generated for lobed and unlobed leaves in the droughted plant and its companion control plant and reported in Table 1 - V. The photosynthetic, transpiration, and stomatal resistance means of the two plants were clearly different as was to be expected.

Single ANOVAS comparing lobed and unlobed leaves were performed for each tree separately using photosynthesis, transpiration, and stomatal resistance as response variables (Table 1- VI).

Table 1 - V. Descriptive statistics for N=216 observations (72 leaves, 3 readings per leaf). Mean and standard deviation for lobed and unlobed leaves measured for photosynthesis (µm per m^2s^1), transpiration (moles per m^2s^1), stomatal resistance (s cm^1), and WUE (water use efficiency).

N	Lobe Category	Treatment	Photosynthesis	Transpiration	Stomatal Resistance	Water Use Efficiency
54	Unlobed	Not droughted	12.486 (4.519)	4.390 (1.633)	2.087 (3.328)	2.867 (0.475)
51	Lobed	Not droughted	13.271 (3.749)	5.601 (1.482)	0.731 (0.487)	2.407 (0.548)
54	Unlobed	Droughted	5.815 (3.152)	1.087 (0.500)	8.207 (7.499)	5.649 (2.585)
57	Lobed	Droughted	6.142 (2.911)	1..255 (0.597)	6.836 (5.499)	5.541 (2.535)

Table 1 - VI. ANOVA results. Power is calculated at 0.05. N represents both group numbers.

Source of variation	DF	SS	F	P	Treatment	Power	N L/U
Photosynthesis							
Lobed/unlobed	1	16.15	0.93	0.3365	Watered	0.15	51/54
Lobed/unlobed	1	2.957	0.30	0.5816	Droughted	0.08	57/54
Transpiration							
Lobed/unlobed	1	38.46	15.77	<0.0001	Watered	0.99	51/54
Lobed/unlobed	1	0.79	2.28	0.1410	Droughted	0.34	57/54
Stomatal Resistance							
Lobed/unlobed	1	48.23	8.29	0.0048	Watered	0.83	51/54
Lobed/unlobed	1	26.40	.67	0.4143	Droughted	0.18	57/54
Water Use Efficiency							
Lobed/unlobed	1	5.56	21.22	<0.0001	Watered	0.99	51/54
Lobed/unlobed	1	0.005	0.001	0.9788	Droughted	0.05	57/54

In the freely-watered plant, lobed leaves had a higher mean for photosynthesis but without statistical significance. The lobed leaves had a higher transpiration mean as well with high statistical significance represented by a P value of 0.0001. The expected lower mean for lobed leaves in stomatal resistance showed high statistical significance with a P value of 0.005. Unlobed leaves had a significantly higher WUE score, and were more distally located on the shoot.

The lobed leaves of the droughted plant had higher means for photosynthesis and transpiration and a lower mean in stomatal resistance, but without statistical significance in any of the categories of response variable (Fig.1- 9). In this droughted plant unlobed leaves were significantly more likely to be further out on the shoot than lobed leaves. ANCOVAS were performed with the added effects of PAR and leaf temperature. P_{net} for the droughted plant had an overall P of <0.0001 but leaf temperature alone accounted for the significance. A single ANOVA testing leaf temperature for lobed and unlobed leaves on this plant indicated no difference between the two means (F = 0.83, P = 0.36, v1, v2 = 1,111). For transpiration, all three effects contributed to the overall significance of the test (P <0.0001). In stomatal resistance, it was again all three effects contributing to the P of <0.0001. ANCOVA for the freely-watered plant with P_{net} as the response variable was overall significant but the lobing effect was only P = 0.07. With transpiration and stomatal resistance as response variables the tests for ANCOVA were significant in all the categories examined.

Table 1-VII shows the results of single ANOVAS that tested the difference in P_{net}, transpiration, and stomatal resistance means of lobed and unlobed leaves on the droughted plant by date. The tests were made to detect any possible advantage that lobed leaves might have in a water stress situation. The first date examined, 9/5, after the tree had been deprived of any water for nine days, shows an advantage for lobed leaves in terms of P_{net} without the concomitant "cost" of transpiration. On the following three dates of measurement the lobed mean for P_{net} is still higher but no longer statistically significant.

Discussion

In the control (watered) plant the water use efficiency rating for the unlobed leaves is significantly higher than that of the lobed leaves because of the high transpiration rate of lobed leaves. In the droughted plant there is no significant difference in WUE between the two types of leaf over the course of the experiment (Fig.1- 10) because the transpiration rate was only marginally higher for the lobed leaf compared to the unlobed leaf. It could be that lobed leaves in a droughted plant are more valuable to the plant than unlobed leaves. This interpretation is supported by the P_{net} results on the first date that the measurements were taken, when the plant had been droughted for nine consecutive days. The performance of lobed leaves is significantly higher than that of unlobed leaves, with a strong Rsq (0.41) to back it up (Table 1-VII).

Table 1 - VII. ANOVA results for droughted plant only, by date. Power is calculated at 0.05. N represents both group numbers. Degrees of freedom is 1 throughout.

Source of Variation	N L/U	F	P	R2	Unlobed Leaf Mean	Lobed Leaf Mean
Photosynthesis	- micromoles per square meter per second					
9/5	6/9	9.15	0.0098	0.41	2.77	3.66
9/11	15/15	0.33	0.5719	0.01	6.57	6.96
9/12	15/15	1.59	0.2172	0.05	3.04	3.90
9/19	18/18	0.0006	0.9809	0.00	8.54	8.57
Transpiration	- millimoles per square meter per second					
9/5	6/9	0.002	0 .9676	0.00	0.44	0 .43
9/11	15/15	3.36	0.0773	0.11	1.37	1.65
9/12	15/15	12.53	0.0014	0.31	0.74	1.06
9/19	18/18	0.37	0.5480	0.01	1.41	1.50
Stomatal Resistance	-centimeters per second					
9/5	6/9	10.3540	0.0067	0.13	22.48	17.39
9/11	15/15	1.1246	0.2980	0.04	4.73	4.23
9/12	15/15	7.6634	0.0099	0 .21	10.63	7.56
9/19	18/18	0.0007	0.9798	0.00	3.13	3.13

SECTION 4

Materials and Methods

This experiment used a mature Morus alba street tree on Goulden Avenue in the Bronx, bordered by a sidewalk on one side and by a field on the other three sides.
A core sample of the tree extracted by an increment bore shows it to be 25 years old. The dbh of the tree is 26 cm. Branches used in this experiment were barely two feet above the ground allowing for easy access to the leaves. The primary shoots used in the experiment were between nine and 26 nodes in length. The leaves measured were from the youngest third of a shoot. Equal numbers of lobed and unlobed leaves were selected. Table 1-VIII records the location for each of the leaves used in this experiment and provides a formula-generated location score (nodal position/total nodes) for each leaf.

Continuous measurements were made with a LI-COR 6200 infra-red gas analyzer on each leaf over a period of approximately one minute. Computed results gave net photosynthetic rate (P_{net}), transpiration rate, and stomatal resistance computed from raw data registered in the chamber. Leaf temperature in the cuvette, and incident PAR were also recorded. The area of each leaf measured was four cm^2. No major veins were present in the leaf section. The sample size was 144, consisting of three measurements of each of 48 leaves (Table 1-IX). The observations were made in late morning or at midday throughout July 1999, an unusually hot month; 11 days of over 90^0 were recorded (NOAA, National Climatic Data Center). The leaves that are classified as shade

Table 1-VIII. Leaf location and Nodal Position Score (NP/TN) for 47 leaves. NP is nodal position and TN is total number of leaves on the shoot. Duplicates represent measurements of same leaf on different days.

Leaf #	# Lobes	NP	NP/TN	TN	Site
1	0	17	0.77	22	shade
2	0	17	0.77	22	shade
3	0	16	0.70	23	shade
4	0	16	0.70	23	shade
5	0	19	0.86	22	shade
6	0	19	0.86	22	shade
7	0	18	0.78	23	shade
8	0	18	0.78	23	shade
9	1	20	0.87	23	shade
10	1	20	0.87	23	shade
11	1	19	0.83	23	shade
12	2	17	0.74	23	shade
13	2	17	0.74	23	shade
14	3	15	0.65	23	shade
15	3	15	0.65	23	shade
16	0	7	0.54	13	sun
17	0	8	0.57	14	sun
18	0	14	0.93	15	sun
19	0	8	0.47	17	sun
20	0	9	0.90	10	sun
21	0	16	0.84	19	sun
22	0	10	0.53	19	sun
23	0	14	0.78	18	sun
24	0	17	0.94	18	sun
25	0	17	0.94	18	sun
26	0	8	0.62	13	sun
27	0	16	0.94	17	sun
28	0	10	0.59	17	sun
29	0	14	0.93	15	sun
30	1	17	0.68	25	sun
31	1	16	0.89	18	sun
32	1	16	0.89	18	sun
33	1	17	0.68	25	sun
34	1	14	0.74	19	sun
35	2	12	0.92	13	sun
36	2	13	1.00	13	sun
37	2	12	0.92	13	sun
38	2	12	0.92	13	sun
39	2	17	0.65	26	sun
40	2	12	0.71	17	sun
41	2	19	0.76	25	sun
42	2	8	0.89	9	sun
43	2	15	0.83	18	sun
44	2	15	0.83	18	sun
45	3	13	0.72	18	sun
46	3	10	0.59	17	sun
47	4	15	0.79	19	sun

Table 1 - IX. Descriptive statistics for N=144 observations (48 leaves, 3 readings per leaf). Mean and standard deviation for unlobed and all-lobed leaves, and for 2-lobed leaves; further categorization by breakdown of site, all measured for photosynthesis (μm m^2s^1), transpiration (mm m^2 s^1), stomatal resistance (c s^1), WUE, and PAR (μm m^2s^1).

N	Lobe	Site	Photosynthesis	Transpiration	Stomatal Resistance	WUE	PAR
66	Unlobed	Sun & Shade	12.55 (5.94)	5.10 (2.14)	1.88 (.93)	2.80 (1.77)	661.81 (574.25)
78	All-lobed	Sun & Shade	15.64 (7.20)	6.35 (3.70)	1.60 (1.01)	3.26 (2.52)	796.72 (607.04)
36	2-lobed	Sun &	17.66 (7.30)	7.13 (3.31)	1.54 (1.20)	3.10 (2.30)	1059.78(584.20)
42	Unlobed	Sun	16.03 (4.60)	5.60 (2.42)	1.81 (1.00)	3.44 (2.00)	954.80 (530.00)
57	All-lobed	Sun	18.90 (5.53)	7.03 (4.10)	1.60 (4.10)	3.90 (2.70)	1026.00(555.00)
30	2-lobed	Sun	20.02 (5.34)	7.70 (3.40)	1.52 (1.30)	3.40 (2.40)	1239.01(461.00)
24	Unlobed	Shade	6.50 (1.10)	4.20 (1.20)	2.02 (1.00)	1.70 (0.52)	149.15 (16.80)
21	All-lobed	Shade	7.00 (2.00)	4.50 (1.00)	2.00 (0.52)	1.60 (0.50)	174.51 (21.64)
6	2-lobed	Shade	5.90 (1.82)	4.50 (1.10)	1.64 (0.16)	1.40 (0.54)	163.67 (6.60)

leaves were western-oriented sun leaves that were measured before the sun was on them. Nonetheless, their computed means in photosynthesis were highly significantly different from sun leaves so they were categorized as a separate classification. Statistical analyses of the data (Goulden Master file) were conducted using Statview 5.0.1 and JMP 3.1.5 on a G3 Power Macintosh.

Results

Descriptive statistics were generated for unlobed leaves, for all lobed leaves viewed as a category, and for two-lobed leaves, in sun and shade together and for each treatment separately (Table 1-IX). The two-lobed leaves were singled out as a separate group for investigation because preliminary analyses indicated that it was within that group that all significant differences between lobed and unlobed leaves lay. One-lobed, 3-lobed, and the single 4-lobed leaf registered no significant differences on any level when each of these groups was compared singly against unlobed leaves.

Single ANOVAS were performed using P_{net}, transpiration, stomatal resistance and WUE (water use efficiency) as response variables. In the initial analyses that included sun and shade leaves, both P_{net} and transpiration showed statistically significantly higher means for all lobed leaves compared to unlobed leaves, and for 2-lobed vs. unlobed leaves (Table 1 - X). To test for possible microenvironmental differences ANOVA was performed with PAR , leaf temperature, and NP/TN score as response variables. PAR

Table 1 - X. ANOVA results. Twenty four separate one-way tests. Power is calculated at 0.05. N shows both group numbers.

Source of variation	DF	SS	F	P	R2	Power	N
<u>Photosynthesis</u>							
Lobed/Unlobed sun & shade	1	341.9	7.75	0.0061**	0.05	0.79	78/66
Lobed/unlobed sun	1	195.3	7.35	0.0080**	0.07	0.77	57/42
Lobed/unlobed shade	1	2.0	1.07	0.3060	0.02	0.16	21/24
2-lobed/Unlobed sun & shade	1	662.0	7.99	0.0006***	0.14	1.00	36/6
2-lobed/Unlobed sun	1	278.0	11.51	0.0011**	0.14	1.00	30/42
2-lobed/Unlobed shade	1	2.0	1.10	0.3027	0.04	0.20	6/24
<u>Transpiration</u>							
Lobed/Unlobed sun & shade	1	58.5	6.18	0.0141**	0.04	0.69	78/66
Lobed/unlobed sun	1	52.2	4.35	0.0397**	0.04	0.04	57/42
Lobed/unlobed shade	1	0.9	0.77	0.3849	0.02	0.14	21/24
2-lobed/Unlobed sun & shade	1	127.9	9.39	0.0002***	0.16	0.98	36/66
2-lobed/Unlobed sun	1	77.5	9.59	0.0028**	0.12	0.86	30/42
2-lobed/Unlobed shade	1	.4	0.31	0.5819	0.01	0.08	6/24
<u>Stomatal Resistance</u>							
Lobed/Unlobed sun & shade	1	3.0	3.15	0.0780	0.02	0.42	78/66
Lobed/unlobed sun	1	1.4	1.25	0.2669	0.01	0.20	57/42
Lobed/unlobed shade	1	1.3	2.44	0.1253	0.05	0.33	21/24
2-lobed/Unlobed sun & shade	1	3.4	1.62	0.2028	0.03	0.34	36/66
2-lobed/Unlobed sun	1	1.5	1.21	0.2755	0.02	0.19	30/42
2-lobed/Unlobed shade	1	0.7	1.16	0.2908	0.04	0.18	6/24
<u>WUE</u>							
Lobed/Unlobed sun & shade	1	7.7	1.59	0.2093	0.01	0.24	78/66
Lobed/Unlobed sun	1	4.0	0.77	0.3814	0.01	0.14	57/42
Lobed/Unlobed shade	1	0.04	0.14	0.7071	0.003	0.06	21/24
2-lobed/Unlobed sun & shade	1	1.6	0.41	0.5239	0.004	0.09	36/66
2-lobed/Unlobed sun	1	0.04	0.01	0.9221	0.000	0.05	30/42
2-lobed/Unlobed shade	1	0.4	1.60	0.2158	0.02	0.23	6/24

showed significant differences between 2-lobed and unlobed leaves in both sun and shade situations. Leaf temperature difference between the all-lobed leaves and unlobed leaves, and between two-lobed and unlobed leaves was significant for sun leaves. The all-lobed mean was higher; the two-lobed mean was higher. The latter had an F of 8, a P of 0.008, and an Rsq of 0.11. ANOVA using NP/TN score as the dependent variable uncovers no significance difference for shade leaves in either the all-lobed/unlobed group or the 2-lobed/unlobed group, or for all-lobed/unlobed sun leaves; there was a significant difference between 2-lobed/unlobed sun leaves which had an F of 6.16 and a P value of 0.02.

There were no significant differences in stomatal resistance on any lobed/unlobed level of examination for sun and shade leaves examined separately in one-way ANOVA.
A breakdown by site showed that for shade leaves overall, there was no statistically significant difference seen in any category of examination save PAR.

<u>All-lobed/unlobed leaves in sun</u>

Sun leaves examined separately in one-way ANOVA showed highly significant differences ($F = 7.3$, $v1,v2 = 57,42$, $P = 0.008$) between lobed and unlobed leaves in P_{net} activity. The mean for unlobed leaves was 16; for all lobed leaves it was 19. The results for transpiration ($F = 4.3$, $v1,v2 = 57,42$, $P = 0.04$) with an unlobed mean of 5.6 and a lobed mean of seven were significantly different (Table 1- X). When P_{net} was tested with PAR and leaf temperature as covariates in an ANCOVA the F was 25, the P value

0.0001, the Rsq 0.44. With transpiration, PAR and leaf temperature as covariates in an ANCOVA the F was 47, the P value 0.0001 and the R–sq 0.60. were significant. PAR were significant. When a MANOVA was performed including P_{net}, transpiration, stomatal resistance, leaf temperature and PAR (F=3, P=0.01), it was seen that P_{net} and PAR were equally important in separating out the multivariate means.

2-lobed/unlobed leaves in sun

Two-lobed sun leaves versus unlobed sun leaves examined in one way ANOVA also showed highly significant differences in P_{net} and transpiration, greater than that found in the all-lobed/unlobed groups (Table 1 - X). The two-lobed leaves had the higher mean in both tests. When ANCOVA was performed on this group for P_{net} with PAR, and leaf temperature as covariates, all of the effects in the model were significant; the whole model F was 15, the P value 0.0001; the Rsq. 0.40. ANCOVA results for transpiration with the same covariates had an F of 24, a P value of 0.0001 and an Rsq of 0.52 with PAR as the only effect having significance. When a MANOVA was performed including P_{net}, transpiration, stomatal resistance, leaf temperature and PAR (F=4, P=0.002), stomatal resistance and PAR were most important in separating out the multivariate means.

Correlation between incident PAR and P_{net}, transpiration, stomatal resistance was always stronger for 2-lobed leaves vs. unlobed leaves in sun. There was a significant relationship between increasing leaf temperature and P_{net}, and transpiration for unlobed sun leaves; for the categories all-lobed and 2-lobed no correlation was seen. Stomatal resistance showed a significant negative association between these variables for unlobed leaves, and no relationship when the categories all-lobed and 2-lobed were examined (Table 1 – XI).

Discussion

There are significant differences in the physiological responses of lobed and unlobed sun leaves. Two-lobed sun leaves have the much higher mean over unlobed leaves in the measurement of photosynthesis (Table 1 - X). They also transpire significantly more. The water use efficiency ratios generated from these two response variables, a mean of 3.44 for unlobed leaves and 3.40 for 2-lobed leaves, show no significant difference. One can conclude from this complete lack of WUE significance that the carbon production of two-lobed leaves is enough to offset their H_2O loss in transpiration and that, compared to unlobed leaves, they are the more productive.

The lack of significant differences in physiological responses for all-lobed vs. unlobed shade leaves is still indirectly informative of how lobed leaves differ from unlobed leaves by site. Lobed leaf P_{net} response in both sun and shade is closely tied to increasing PAR. Unlobed leaves show this correlation only in sun (Fig.1-11). Lobed leaf transpiration rates correlate significantly with increasing PAR in sun but not in shade; for unlobed leaves there is no correlation in shade. Stomatal resistance of lobed sun leaves

Table 1- XI. Photosynthesis, Transpiration & Stomatal Resistance correlation to PAR and to Leaf Temperature in the cuvette for sun leaves.

N	Leaf Type	Correlate	Photosynthesis		Transpiration		Stomatal Resistance	
			Correlation	P value	Correlation	P value	Correlation	P value
42	Unlobed	PAR	0.408	0.0073	0.657	0.0001	-0.192	0.224
57	All-lobed	PAR	0.741	0.0001	0.827	0.0001	-0.500	0.0001
30	2-lobed	PAR	0.631	0.0002	0.726	0.0001	-0.452	0.012
42	Unlobed	Lf. temp.	0.428	0.002	0.529	0.0001	-0.432	0.002
57	All-lobed	Lf. temp.	0.077	0.784	-0.202	0.471	0.341	0.214
30	2-lobed	Lf. temp.	0.062	0.744	0.318	0.09	-0.144	0.448

decreases significantly as PAR increases; not so for lobed shade leaves. Unlobed leaves show no significant correlation between PAR and stomatal resistance in sun or shade. Given that leaf specific mass is not different between lobed versus unlobed leaves, this sensitivity on the part of lobed leaves to PAR must be due to varying biophysical, heat transfer performance and/or to different, internal physiology of the two leaf types. The N of the two-lobed shade leaf was only 6 as against 24 unlobed shade leaves. It may be that a larger (Power analysis LSN 106) and more equally distributed sample size is needed to detect statistical differences.

Looking once more at direct lobed vs. unlobed leaf comparisons, the stomatal resistance measurements showed no statistically significant differences between unlobed and all-lobed leaves or between unlobed and two-lobed leaves in sun and shade together or separately. Nonetheless, the unlobed mean was always higher than the lobed means. Stomatal resistance may be related to the statistically significantly higher stomate count for unlobed leaves in both sun and shade shown in a separate experiment reported in Chapter 2.

SECTION 5

Material and Methods

Four Morus alba plants were used in this experiment. Two of them were potted plants that had been used in an earlier droughting experiment; both were growing in a permanent shade enclosure. Two were in-ground plants; one growing in full sun, one in a permanent shade enclosure.

The measurements were made on a clear, sunny day at midday in the month of October. From each plant five lobed leaves and five unlobed leaves were selected. Petiole length and internodal distance to the subtending leaf were recorded prior to removal from the shoot. Upon removal, the petiole was trimmed and the leaf placed in a Scholander chamber (Scholander et al. 1965). Using delivery pressure of 250 psi, leaf xylem water potential was measured in bars at the second that xylem bubbling was observed on the petiole tip. The resulting data were entered in Statview (ver.5.0.1).

Analyses that follow were generated in computer file "Drought exp. 12/13, 216 rows."

Results

The mean and standard deviation are provided for each of the four treatments in Table 1 –XII as are the results of one-way ANOVA testing the Scholander chamber readings. The potted control plant had a significant difference between lobed and unlobed leaves (P=0.02) and the in-ground sun plant was very close to significance (P=0.06). In the potted control plant, the unlobed leaves had the greater negative water potential. In the sun plant, it was the lobed leaves that had the greater negative water potential.

Table 1 - XII. Descriptive statistics and ANOVA results for leaf water potential means. Leaf water potential measured in -Bars.

Treatment	N L/U	F	P	R^2	Leaf Water Potential Mean (±SD)	
					Lobed	Unlobed
Watered potted, in shade	5/5	8.46	0.02	0.51	2.85 (±1.06)	6.00 (±2.18)
Droughted potted, in shade	5/5	0.14	0.91	0.00	2.45(±0.62)	2.50 (±0.71)
Sun inground	5/5	4.89	0.06	0.38	6.90 (±3.58)	3.30(±0.67)
Shade inground	5/6	.002	0.97	0.00	3.50 (±0.94)	3.46 (±1.87)

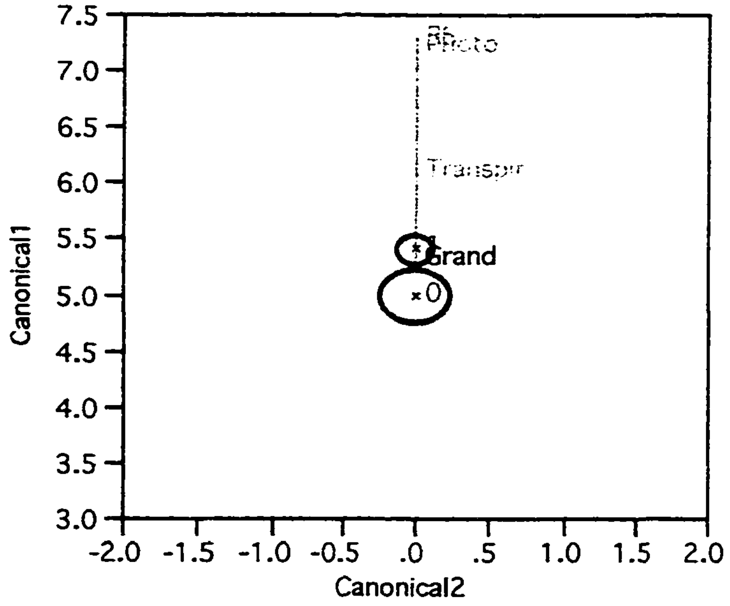
Regression analysis disclosed a positive significant relationship for unlobed leaves between the petiole length of the droughted plant leaves and the Scholander reading There was near significance (P=0.06) for the negative relationship of these same two variables for lobed leaves in the control (watered) plant (Fig. 1 -12). The in-ground shade plant also had near significance (P=0.07) for the negative relationship between internodal distance and the Scholander reading for unlobed leaves.

Discussion

The lobed leaf of the in-ground sun plant was more stressed than the unlobed leaf (Table 1-XII). This was not an unexpected result given the lower stomatal resistance and higher transpiration rate of lobed leaves in the preceding experiments. The reverse situation in the potted plant – the unlobed leaf is more stressed - that had served as the control in an earlier droughting experiment is not easily explained for just the same reasons. One would be tempted to ascribe these contradictory results to small sample size were it not for the robust R squares associated with the results. ANOVAS that examined the difference between lobed/unlobed leaves in the four treaments for petiole length and for internodal distance were not significant. The regression analysis indicated a relationship between greater stress and longer petioles for unlobed leaves in the droughted plant; in the control plant the relationship was between greater stress and shorter petioles for lobed leaves.

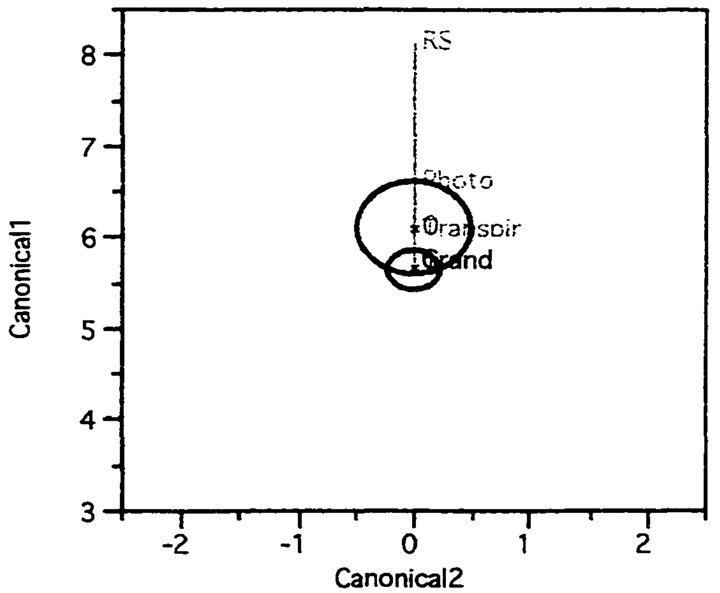
Figure 1-1. Canonical centroid plots generated in Manova for unlobed leaves and all-lobed leaves in sun and in shade.

	F	v1	v2	P
Wilks' Lambda	3.1288	3	284	0.0261



Unlobed/all lobed SUN

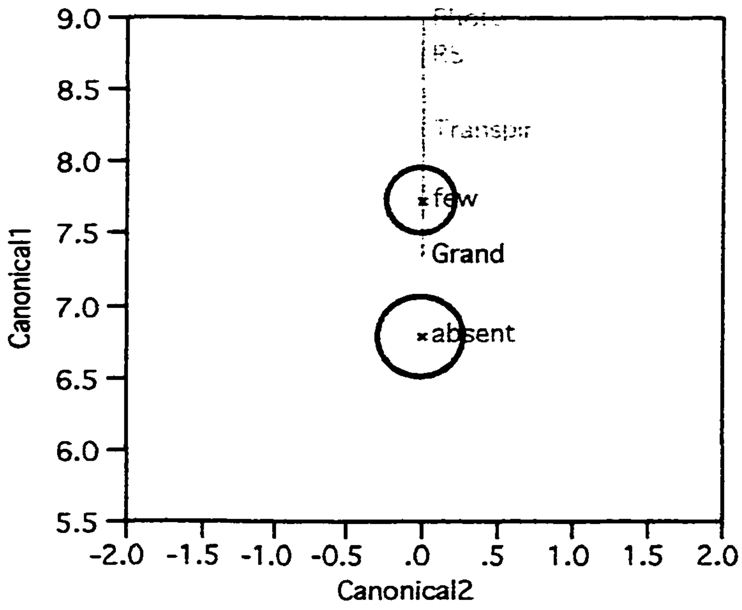
	F	v1	v2	P
Wilks' Lambda	0.8584	3	89	0.4658



Unlobed/all lobed SHADE

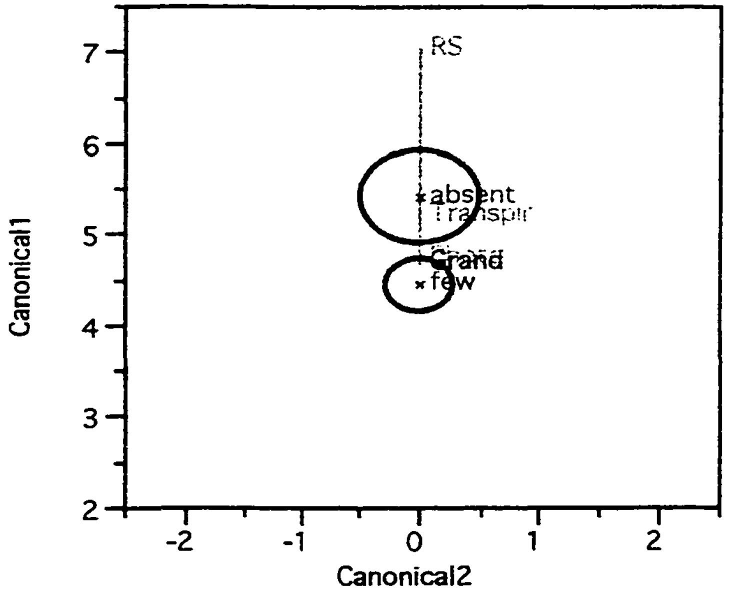
Figure 1- 2. Canonical centroid plots generated in Manova for unlobed leaves and few-lobed leaves in sun and in shade.

	F	v1	v2	P
Wilks' Lambda	8.6231	3	119	<.0001



Unlobed/few-lobed SUN

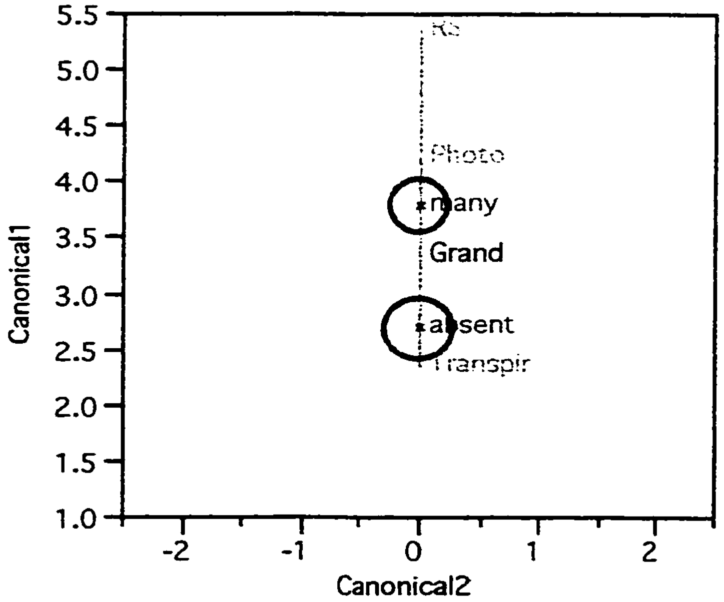
	F	v1	v2	P
Wilks' Lambda	3.4097	3	59	0.0232



Unlobed/few-lobed SHADE

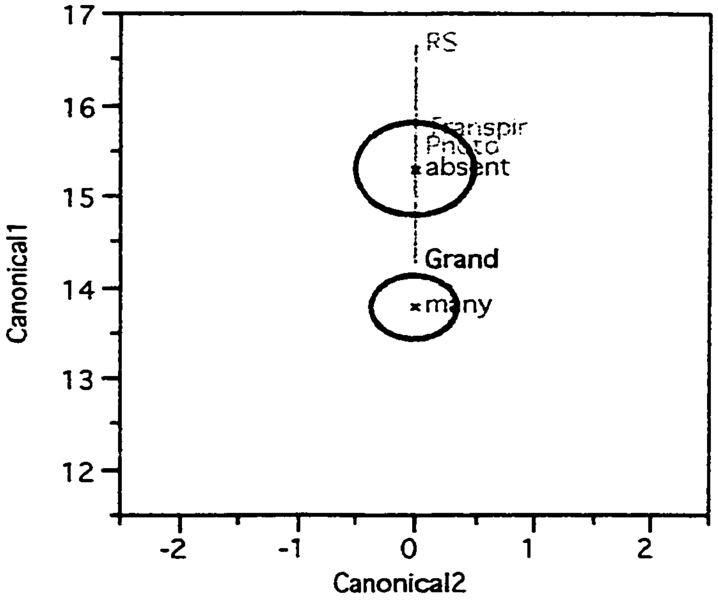
Figure 1- 3. Canonical centroid plots generated in Manova for unlobed leaves and many-lobed leaves in sun and in shade.

	F	v1	v2	P
Wilks' Lambda	11.8596	3	119	<.0001



None/Many SUN

	F	v1	v2	P
Wilks' Lambda	7.2423	3	41	0.0005



None/many SHADE

Figure 1 - 4. Scatterplots and correlation analyses for selected response variables versus PAR for sun and shade leaves graphed with 95% prediction ellipses of the points.

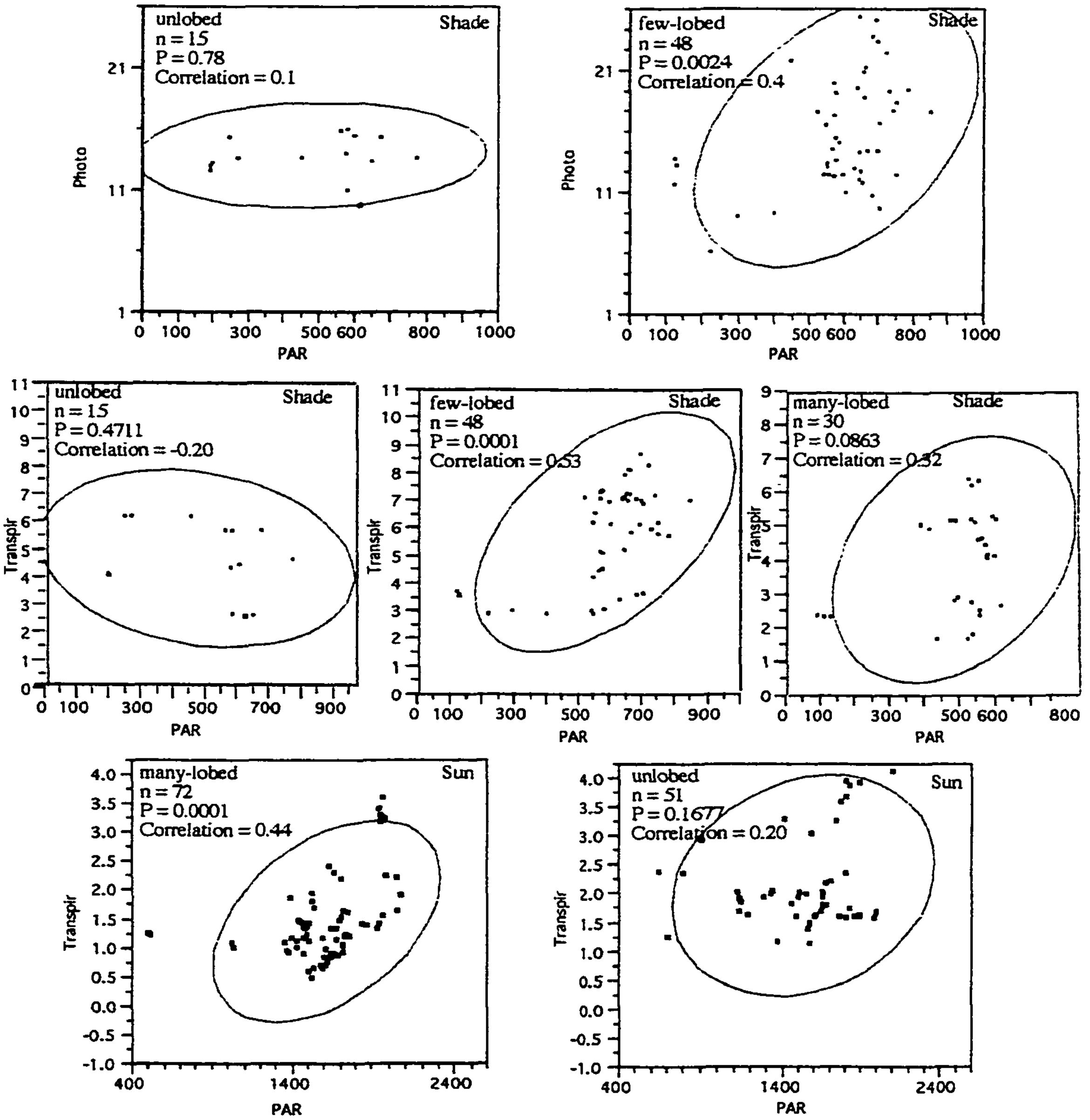
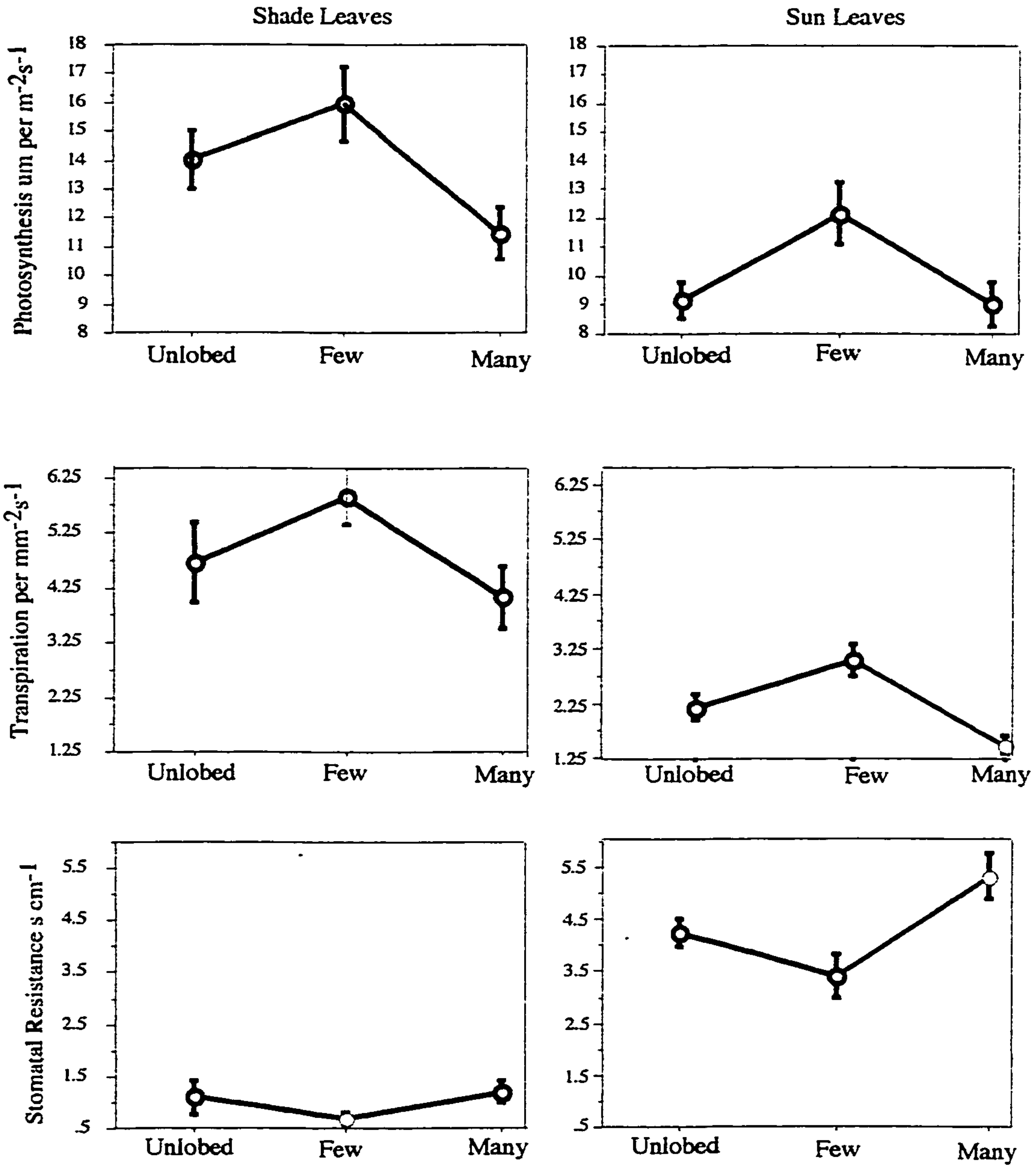


Figure 1- 5. Comparative means for photosynthesis, transpiration, and stomatal resistance for unlobed, few-lobed,and many-lobed Morus alba leaves in sun and in 60% shade conditions. 95% confidence intervals for all graphs.



Shade Leaves

Sun Leaves

Figure 1- 6. Comparative means for leaf temperature, PAR, and WUE for unlobed, few-lobed, and many-lobed Morus alba leaves in sun and in 60% shade conditions. 95% confidence intervals forall graphs.

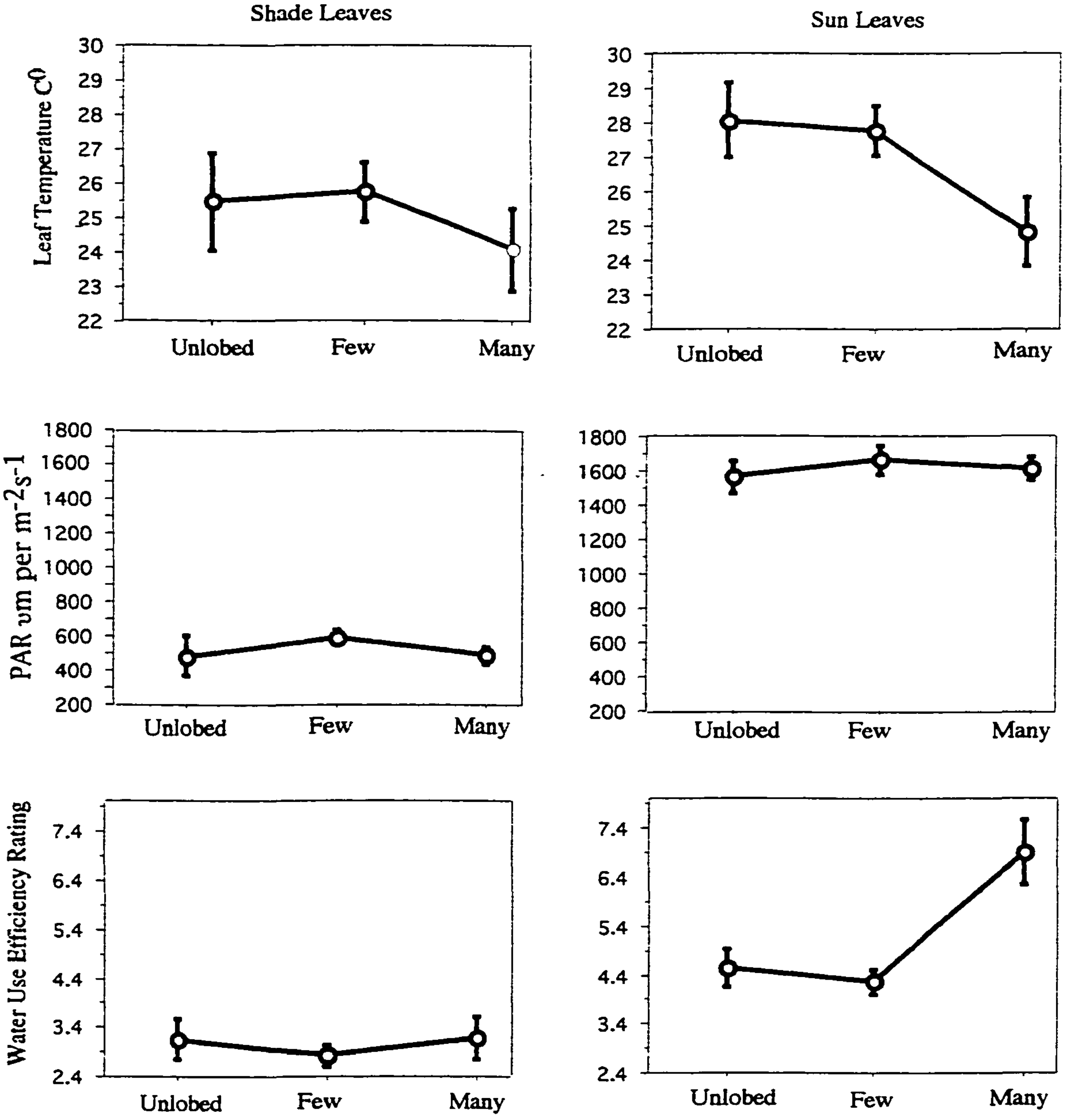
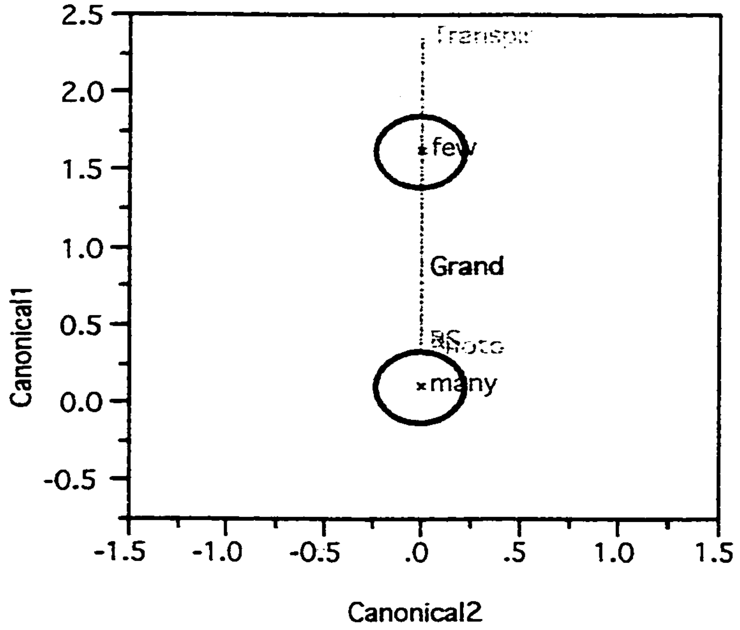


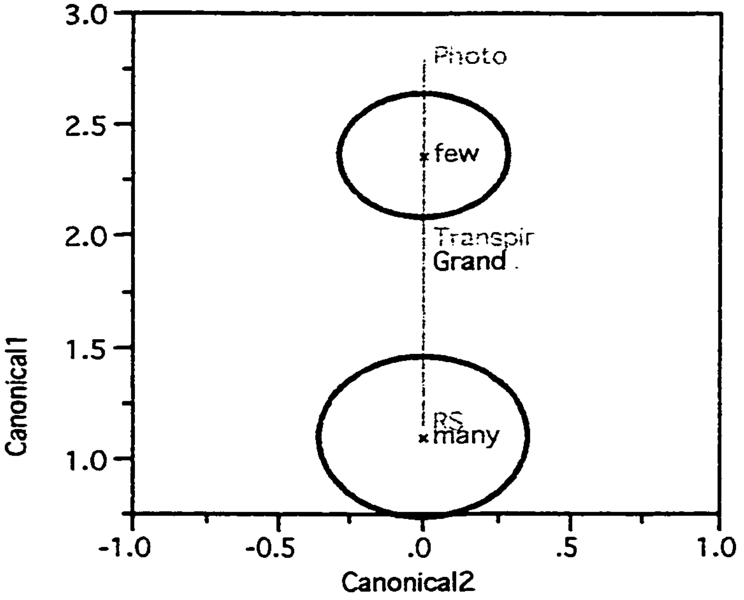
Figure 1-7. Canonical centroid plots generated in Manova for few-lobed leaves and many-lobed leaves in sun and in shade.

	F	v1	v2	P
Wilks' Lambda	27.3743	3	140	<.0001



Few/many SUN

	F	v1	v2	P
Wilks' Lambda	9.6442	3	74	<.0001



Few/many SHADE

Figure 1 - 8. Scatterplots and correlation analysis for P_{net} versus PAR for 2-lobed and unlobed leaves shade graphed with 95% prediction ellipses of the points.

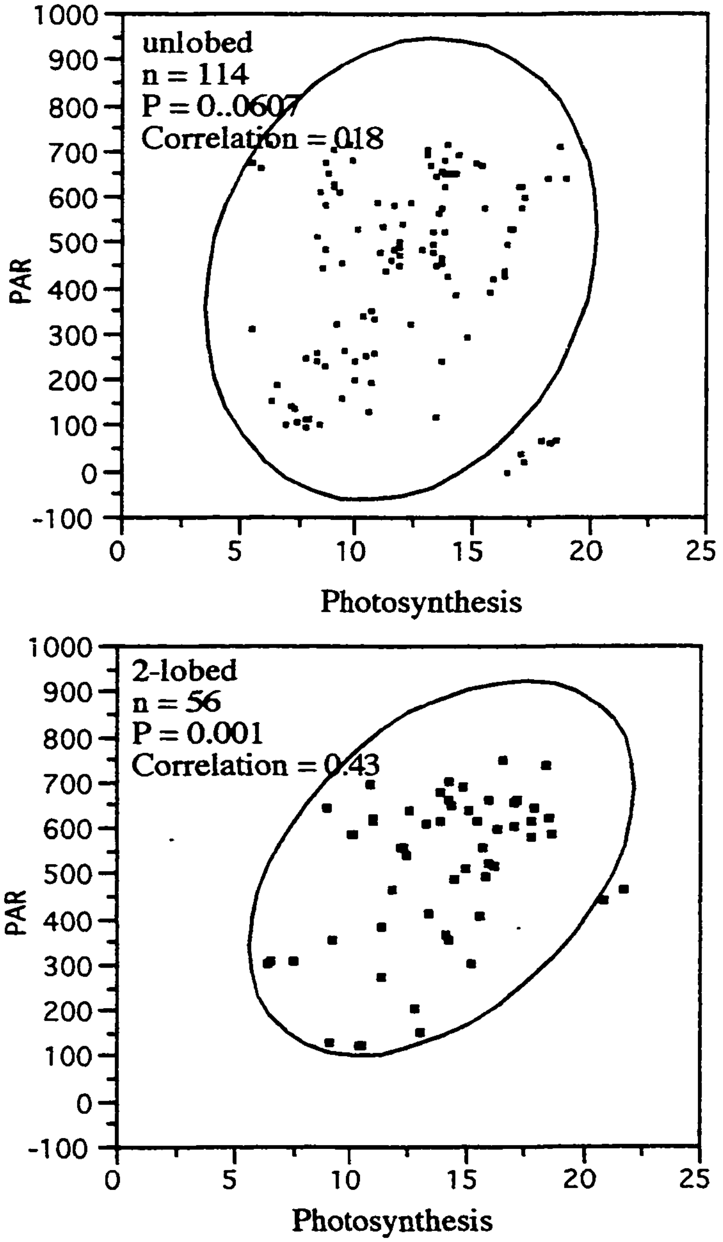


Figure 1- 9. Bar chart of means for lobed and unlobed leaves in tree 12 and tree 13 for photosynthesis, transpiration, stomatal resistance and water use efficiency. An asterisk indicates statistically significant difference (P<0.05) between lobed and unlobed readings for the same tree.

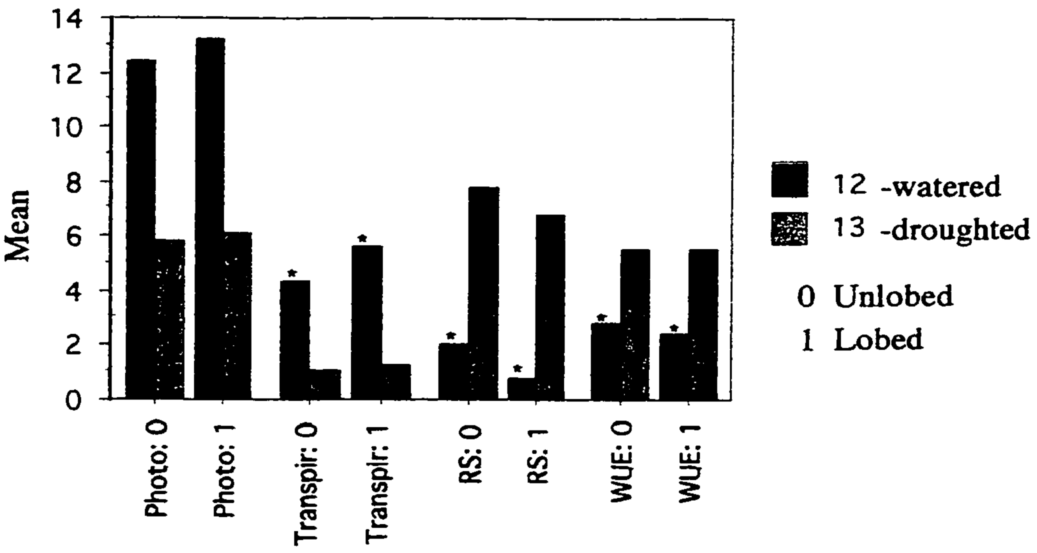
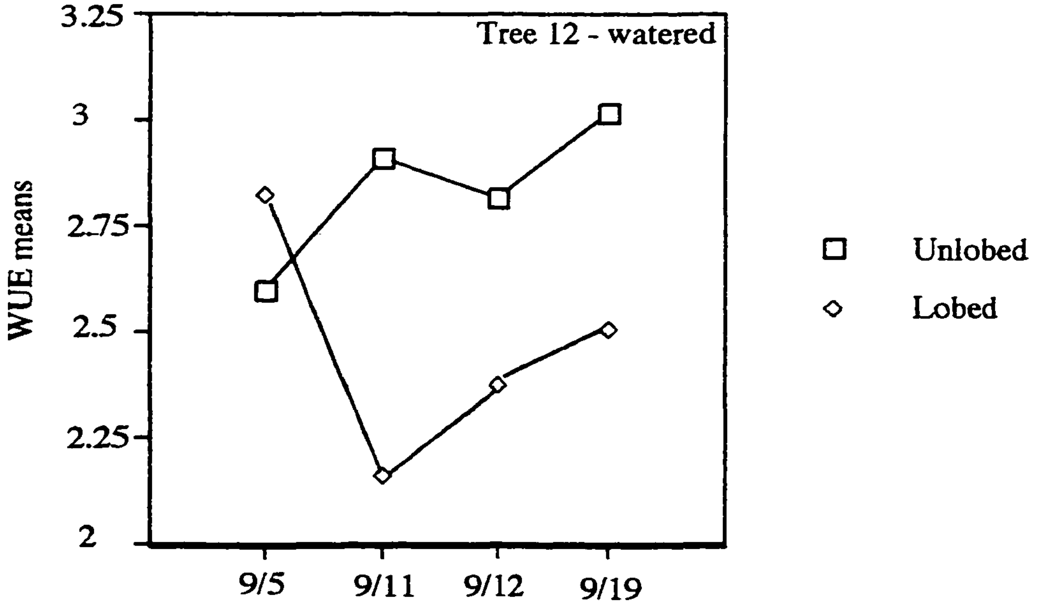


Figure 1-10. Water use efficiency ratings for lobed and unlobed leaves in the droughted plant and the watered control plant, by date. WUE (µm m^2s^1)/(m m^2s^1).



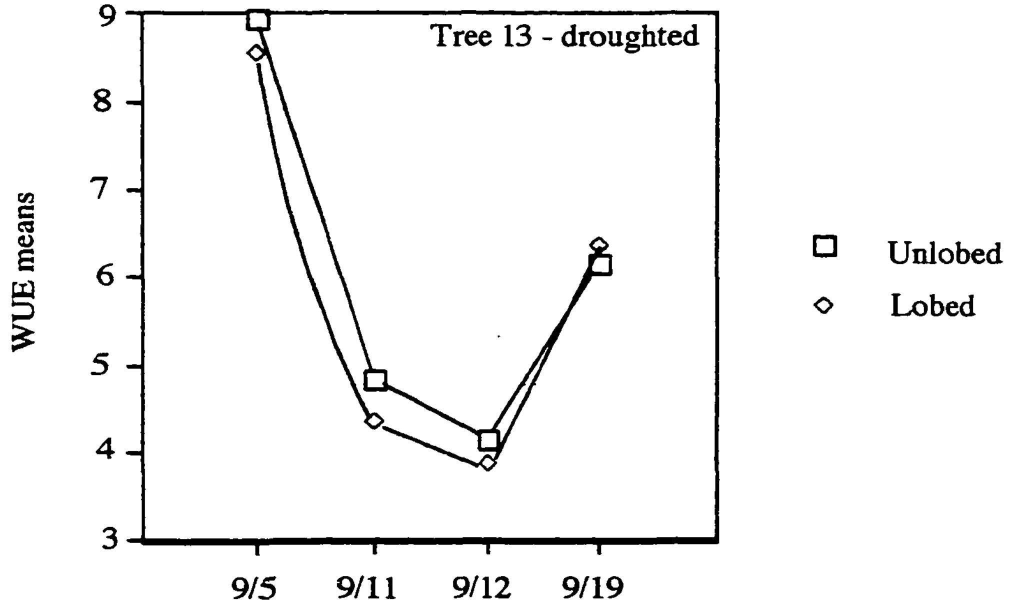


Figure 1 - 11. Scatterplots and correlation analysis for P_{net} versus PAR for lobed and unlobed sun and shade leaves graphed with 95% prediction ellipses of the points.

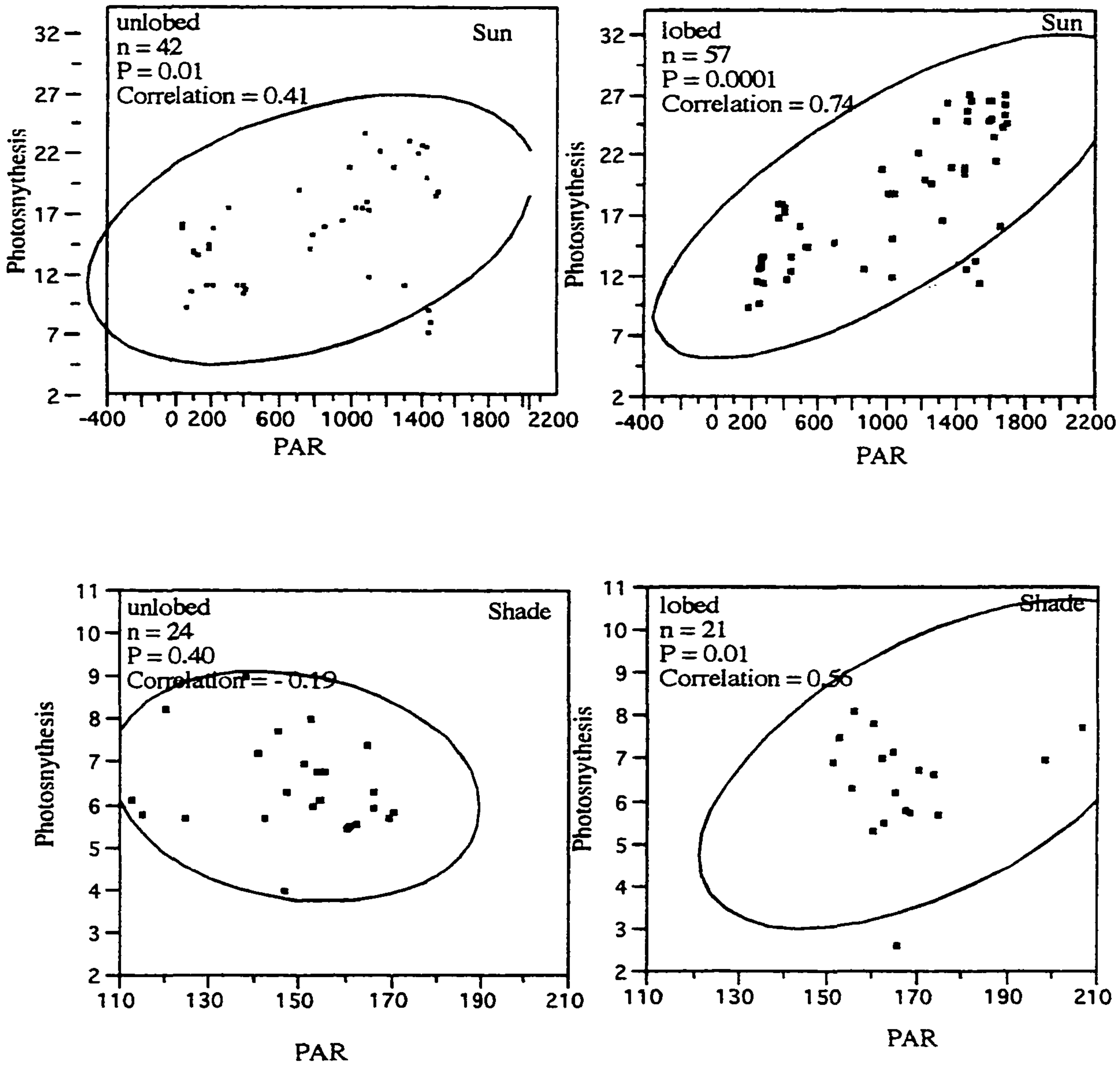
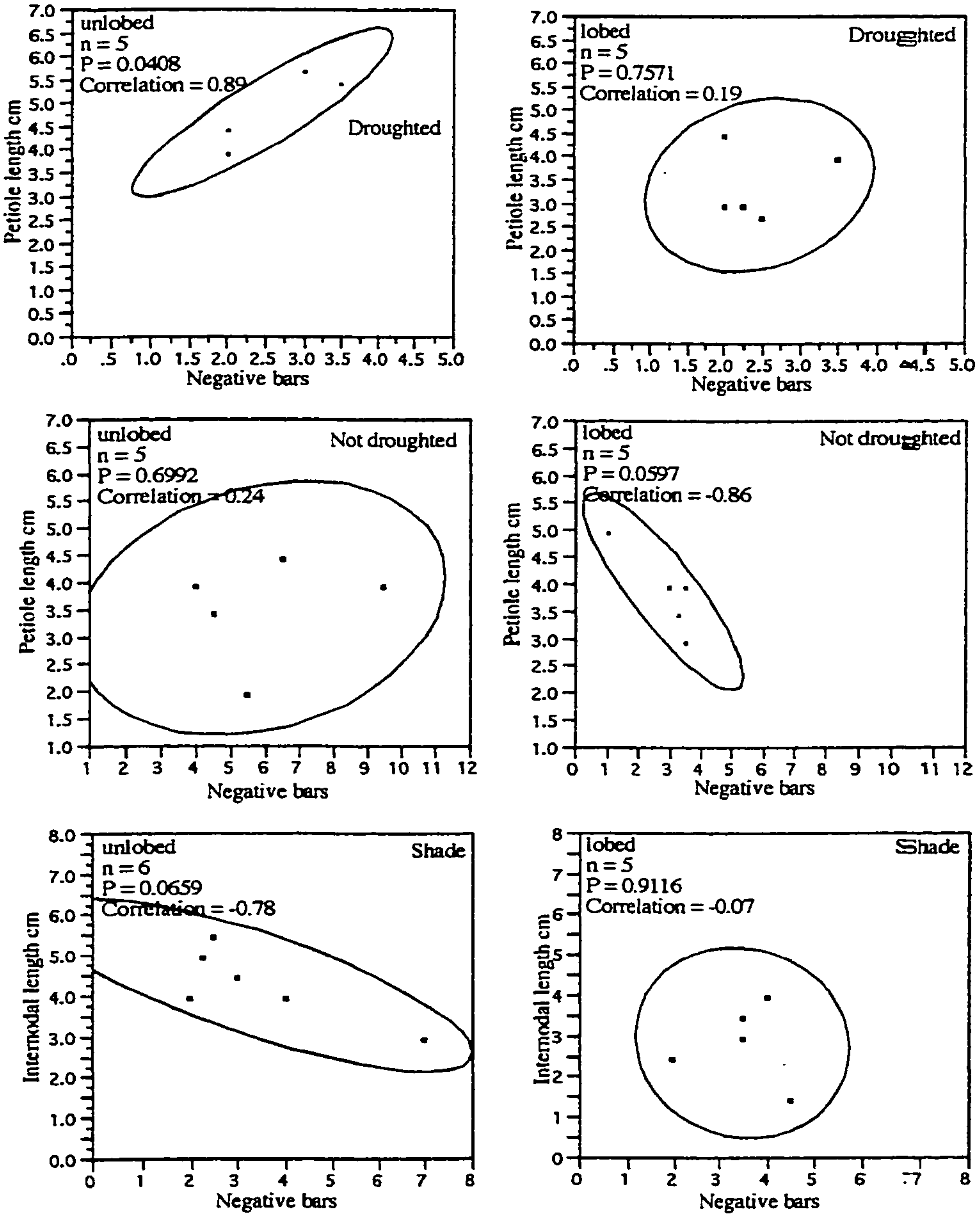


Figure 1- 12. Scatterplots and correlation analyses for selected response variables versus Scholander readings (negative bars) for leaves in different treatments. All graphs have 95% prediction ellipses of the points.



CHAPTER 2

STOMATAL DENSITY AND MEASUREMENTS FOR LOBED AND UNLOBED LEAVES IN DIFFERENT TREATMENTS.

SUMMARY

Eight leaves were used in a study of comparative stomatal density and size. One lobed and one unlobed leaf were taken from a plant in full sun, from one in 60% shade, from one supplied with adequate nitrogen, and from one subjected to severe nitrogen deprivation. Plants were all from cuttings taken 18 months earlier from different street trees. Insertion points of all the leaves were similar.

Two sections were removed from each leaf, one from the center of the blade half, and one from near the midrib. The sections were viewed under a Hitachi S-2700 SEM and 10 micrographs were taken of each section. Stomates on each micrograph were tallied and the means for each micrograph were entered in a Statview 5 data table.

Within a leaf, significant differences in density were found between stomates near the midrib and stomates in the center of the blade half. Significant differences were also found in stomatal density between treatments. Stomatal density was greater in unlobed leaves in the sun, shade, and standard nitrogen treatments; lobed leaves had more stomates in the nitrogen deprivation treatment.

Guard cell length in the full sun plant was significantly greater for the lobed leaf than the unlobed leaf.

Introduction

This chapter investigated stomate density and stomate size in Morus alba. The objective was to uncover any anatomical differences analogous to the physiological variation that was seen in lobed and unlobed leaves. Stomate counts were made using fully-developed lobed and unlobed leaves from in-ground plants in sun and shade situations and from potted plants both with and without nitrogen supplements. Stomate measurements were made from the same leaf sections used for the counts.

Materials and Methods

Plants used in this investigation were grown from cuttings taken 18 months earlier from roadside Morus alba trees in New York City. Eight fully developed Morus alba leaves were used. One lobed and one unlobed leaf were selected from each of the following plants: an in-ground plant in full sun; an in-ground plant growing under shade cloth which allowed only 40% of full sunlight intensity; a glasshouse potted plant provided with standard nutrients (20-20-20) throughout the growing season; a glasshouse potted plant that was nitrogen deprived. The ambient light in the glasshouse was supplemented by high pressure sodium 3,000 watt lights set to a 14-hour day length. The eight leaves were all chosen from similar insertion points on the shoot to minimize differences of stomatal numbers due to nodal location (Wilmer and Fricker 1996, Martin et al. 1983, Salisbury 1928). Since stomatal frequency is not uniform over the surface of a leaf (Wyers and Meidner 1990, Salisbury 1928) two pieces (approx. 5sq.mm.) were excised from each of the eight leaves: one from near the midrib of the leaf and one from a more central part of the lamina (Fig. 2-1). Care was taken to avoid any major veins. Each leaf piece was preserved in 50% ETOH initially and then brought up to 100% ETOH. Each leaf section was divided again to provide an adaxial view and an abaxial one on the stub before critical point drying; each was gold coated in preparation for viewing under an Hitachi S-2700 SEM.

A total of 160 micrographs were taken at a magnification of 733. Each micrograph represented an area of leaf measuring 0.045 sq. mm. (Figs. 2-7,2- 8). Stomatal frequency counts were made by dividing a micrograph into five sections, each section measuring approximately 3 1/2 cm. x 14 1/2 cm. Two manual counts were made of the stomates in each micrograph on two different occasions to allow for possible error in identification of a stomate. Both raw counts were entered into Statview as data, resulting in a data table containing 320 rows. Since my interest was in determining the absolute number of stomates in a given area rather than the proportion of stomates to neighboring epidermal cells, I did not use stomatal index (the number of stomata per unit leaf area divided by the number of stomata per unit leaf area plus the number of epidermal cells per unit leaf area times 100) as devised by E.J. Salisbury (Salisbury 1928) for comparing leaves from different ecological situations.

For stomate measurements, only those stomates that were clearly in focus on the micrograph were used. Four measurements (mm) per stomate were made. These measurements included: outside edge to outside edge of the guard cells, length of the guard cells, aperture width, aperture length (Fig. 2- 4). In their study of stomates, Carpenter and Smith (1975) measured only guard cell length and aperture length. They contended that stomatal width was unreliable. I have included stomatal width after examining the micrographs from the different situations and finding variability throughout, i.e. each group of micrographs showed a range of stomatal openness.

Results

Morus alba is a hypostomatous plant and its stomates are anomocytic. Salisbury (1928) reported that "typically, each square millimeter of leaf surface has about 100 stomates, but the number can be ten times that..." In my study the overall mean stomate count for all four situations (sun, shade, nitrogen, nitrogen-deprived) and both types of leaf (lobed, unlobed) was 36 stomates per 0.045 sq. mm. of viewing area or 800 stomates per sq. mm and 80,000 per sq. cm. This places Morus alba in the highest category of stomatal frequencies according to Wilmer and Fricker (1996) and according to Carpenter & Smith (1975), on a par with Acer rubrum (70,455 sq. cm.), Quercus palustris (90,909 sq.cm.), Quercus coccinea (75,959 sq.cm.), and Tilia americana (89,100 sq.cm.). Stomatal frequencies in Morus alba are higher than Betula spp. (237 sq.mm.) and olive (625 sq.mm.) (Fuller and Tippo1949). All of these stomatal tallies were raw counts, not stomatal indices.

STOMATE COUNTS

There were three levels of variation in the Morus alba stomate counts. Differences were found between stomate figures in the central portion of the lamina and the near- midrib portion. Differences were found between treatments for a single leaf type, i.e. lobed in sun vs. shade or unlobed in sun vs. shade (Fig.2- 9). Differences existed between leaf type per treatment, i.e. lobed or unlobed (Fig.2- 3). Both latter comparisons agree with Wilmer's (1983) findings that stomatal frequency is affected by ecological factors.

Stomate site variation in unlobed leaves

For all four treatments – sun, shade, nitrogen, no nitrogen - the central leaf pieces had higher stomatal density than the near midrib pieces (Tables 2-I through 2- IV) (Fig.2- 2).

Stomate site variation in lobed leaves

In the full sun, shade, and standard nitrogen situations, the count between center and near midrib stomates was almost equal. By contrast, in the nitrogen deprivation situation the lobed leaf's centrally placed stomates far outnumber those in the near midrib portion of the blade (Tables 2- I through 2- IV) (Fig. 2- 2).

Table 2-I. Descriptive statistics and ANOVA results for stomate density in lobed and unlobed leaves of Morus alba grown in full sun.

N	Sun	Position	Mean/SD of stomates .045 sq. mm. leaf area	
20	Lobed	Central	31.55	(5.11)
20	Unlobed	Central	53	(7.74)
20	Lobed	Near midrib	30.8	(4.47)
20	Unlobed	Near midrib	40.5	(9.47)

ANOVA	F	P	Rsq.
Central position			
Lobed vs. unlobed	107	<0.0001	0.52
Near midrib position			
Lobed vs. unlobed	17.2	0.0002	0.31

Table 2-II. Descriptive statistics for stomate density in lobed and unlobed leaves of Morus alba grown under shade cloth cover that excluded 60% of the light.

N	Sun	Position	Mean/SD of stomates .045 sq. mm. leaf area	
20	Lobed	Central	45.00	(3.11)
20	Unlobed	Central	56.15	(7.13)
20	Lobed	Near midrib	39.70	(4.0)
20	Unlobed	Near midrib	39.60	(3.30)

ANOVA	F	P	Rsq.
Central position Lobed vs. unlobed	41.13	<0.0001	0.52
Near midrib position Lobed vs. unlobed	.01	0.93	0.00

Table 2-III. Descriptive statistics and ANOVA results for stomate density in lobed and unlobed leaves of Morus alba supplied with nitrogen.

N	Sun	Position	Mean/SD of stomates .045 sq. mm. leaf area
20	Lobed	Central	29.35 (7.97)
20	Unlobed	Central	35.80 (6.31)
20	Lobed	Near midrib	28.10 (5.02)
20	Unlobed	Near midrib	31.10 (6.55)

ANOVA	F	P	Rsq.
Central position			
Lobed vs. unlobed	8.18	0.0068	0.18
Near midrib position			
Lobed vs. unlobed	2.5	0.1243	0.07

Table 2- IV Descriptive statistics and ANOVA results for stomate density in lobed and unlobed leaves of Morus alba deprived of nitrogen.

N	Sun	Position	Mean/SD of stomates .045 sq. mm. leaf area
20	Lobed	Central	38.8 (5.45))
20	Unlobed	Central	28.6 (3.50)
20	Lobed	Near midrib	23.10 (4.8)
20	Unlobed	Near midrib	25.8 (5.1)

ANOVA	F	P	Rsq.
Central position			
Lobed vs. unlobed	49.67	<0.0001	0.57
Near midrib position			
Lobed vs. unlobed	2.94	0.0945	0.07

Stomate count variation of unlobed leaves in sun vs. shade

The counts for central and near midrib stomates in the unlobed sun leaf were similar to that of the unlobed shade leaf (R sq= 0.003, F = 0.24, P = 0.63, v1,v 2 = 1,78) (Fig. 2- 9). The sun mean was 46.75; the shade mean 47.88. By comparison, Salisbury (1928) reported that sun leaves have a greater number of stomates than shade leaves.

Stomate count variation of lobed leaves in sun vs. shade

The number of stomates in the central and near midrib sections in the shade lobed leaf were significantly greater than those found in the lobed sun leaf pieces (R sq.=.60, F=118, P= <0.0001, v1,v 2=1,78) (Fig.2- 9). The sun mean was 31.18; the shade mean 42.35. This result is in complete contradiction to Salisbury's (1928) finding that sun leaves have a greater number of stomates than shade leaves.

Stomate count variation of unlobed leaves with nitrogen vs. nitrogen deprivation

The unlobed leaf provided with standard nitrogen had significantly more stomates than the unlobed leaf in a nitrogen-deprived situation (R sq.= 0.23, F = 23.2, P <0.0001, v1,v 2 = 1,78) (Fig. 2- 10). The nitrogen mean was 33.38; the nitrogen -deprivation mean was 27.18.

Stomate count variation of lobed leaves with nitrogen vs. nitrogen deprivation

The mean stomate count for the lobed leaf with nitrogen is 28.7; for the lobed leaf with nitrogen deprivation, it is 30.9. The means are similar in this comparison (Rsq.= 0.019, F =1.53, P= 0.220, v1,v 2=1,78) (Fig. 2-10).

Stomate count in lobed vs. unlobed leaves

Descriptive statistics were generated for central and near midrib sections of lobed and unlobed leaves in all four situations - in-ground sun, in-ground shade, nitrogen potted, no nitrogen potted - and reported in Tables 2-I through 2-IV. These tables list the results of single ANOVA tests performed using stomate count as the response variable.

For the in-ground sun plant, the unlobed leaf had highly significantly more stomates in both the central and the near midrib portions compared to the lobed leaf (Table 2-I, Fig.2- 2)

For the in-ground shade plant the central portion of the unlobed leaf had significantly more stomates than the lobed leaf while the near midrib sections of lobed and unlobed had almost identical counts (Table 2- II, Fig. 2- 2).

The central portion of the unlobed leaf from the potted plant with standard nitrogen nutrition had highly significantly more stomates than the lobed leaf. The midrib portion of this unlobed leaf also had more stomates, but the difference was not significant (Table 2- III, Fig. 2- 2).

Stomatal density in the central portion of the lobed leaf of the potted plant that had been deprived of nitrogen was significantly greater than that in the unlobed leaf. In the near midrib portion, the comparison to the unlobed leaf was not significant (Table 2-IV, Fig. 2- 2).

STOMATE MEASUREMENTS

Four measurements were made on each stomate that was clearly visible in the 80 micrographs of the centrally-located leaf segments (10 sun lobed, 10 sun unlobed , 10 shade lobed, 10 shade unlobed, 10 nitrogen lobed, 10 nitrogen unlobed, 10 no nitrogen lobed, 10 no nitrogen unlobed): guard cell length, guard cell width, aperture length, and aperture width (Fig.2- 4, Table 2-V). In separate ANOVA tests of the entire sample, 255 stomates from the lobed leaves and 342 stomates from the unlobed leaves (N = 617) the lobed leaf stomates had longer and wider guard cells with longer and wider apertures than the unlobed leaves; all had low R squares. When the sample was examined by treatment, only the ANOVA for guard cell length in the sun leaves showed true significance (F=102, P <.0001, R sq = 0.39, v1,v 2 =1,159). The lobed leaf mean was 12.67; the unlobed leaf 10.15. Canonical centroid plots generated in MANOVA supported these findings (Figs. 2- 5, 2- 6).

Table 2-V. Means and standard deviations for measurements of 617 centrally-located stomates, in mm.

Lobe Category	Treatment	N	Guard Cell Length	Guard Cell Width	Aperture Length	Aperture Width
Lobed	sun	46	12.67(1.38)	6.05(1.09)	6.37(1.68)	1.49(0.67)
Unlobed	sun	115	10.15(1.45)	5.97(1.01)	6.19(1.34)	1.52(0.71)
Lobed	shade	123	11.36(1.74)	6.57(1.26)	8.20(1.81)	1.93(0.90)
Unlobed	shade	95	10.83(1.14)	6.40(0.92)	7.54(1.31)	1.61(0.70)
Lobed	nitrogen	40	10.20(1.57)	4.90(0.95)	5.29(1.18)	1.02(1.60)
Unlobed	nitrogen	51	10.26(1.25)	5.75(1.11)	5.51(1.49)	0.92(0.51)
Lobed	no nitrogen	60	10.05(1.51)	5.52(0.97)	6.15(1.64)	1.06(0.51)
Unlobed	no nitrogen	87	9.68(1.03)	4.99(0.81)	5.74(1.01)	0.94(0.39)

Discussion

The role of stomates in the function of a leaf is vital. Their number and placement on a leaf surface allow a maximum transfer of CO_2 into the leaf (Bidwell 1974). The literature is replete with references to the fact that stomate numbers differ on leaves within the same plant depending on the insertion point of the leaf. There is unanimity among researchers regarding environmental effects on stomate number in plants of the same species and in leaves of the same plant (Mansfield and Davies 1985). Loftfield (1921) and Martin et al. (1983), comparing entire plants, state that where there are fewer stomates, i.e. in plants grown in shade or in a dry habitat, these stomates are generally larger so that total stomatal pore area is relatively stable. What I have not found in the literature is any reference to stomate numbers and stomate sizes relating to lobed vs. unlobed leaves at similar insertion points on the shoot.

There are unquestionable differences in stomate density between lobed and unlobed leaves on the plant in full sun, one the plant in the 60% shade enclosure, on the potted plant provided with adequate nitrogen, and on the potted plants deprived of nitrogen. Stomatal density was greatest in the unlobed leaf when the plant was in full sun and with adequate nitrogen. The unlobed shade leaf also had overall stomatal numerical superiority but this was somewhat tempered by equality of the near-midrib stomates in lobed and unlobed leaves. The nitrogen deprived lobed leaf had far more central stomates than the unlobed leaf.

A second level of difference was seen between lobed and unlobed leaves based on their respective responses to sun and shade conditions. The lobed leaf produced significantly more stomates in shade than in sun. In the central part of the blade the count was 45 to 32; in the near-midrib section it was 40 to 31. The unlobed leaf showed no difference in stomate numbers for either situation. However, Wilmer and Fricker (1996), Loftfield (1921) and Salisbury (1928) all report that shade leaves contain fewer stomates than sun leaves.

The third level of significant difference in the number of stomates between lobed and unlobed leaves is seen in the stomate location count. Figure 2- 2 illustrates the difference in the ratio of central to near midrib stomates in the nitrogen deprived lobed leaf. Here, the central stomates of the lobed leaf are significantly more numerous than the near midrib stomates of the same leaf; the unlobed leaf shows no difference between the two sites. In 60% shade, central stomates of the unlobed leaf are significantly more numerous than near midrib stomates of the same leaf. The lobed leaf shows no difference between its central and near midrib sites. These results are contradictory as the nitrogen deprivation and shade situations are both environmentally stressful. A rationale for this behavior does not immediately present itself.

Stomatal density and location patterns appear to be random. In sun, shade, and adequate nitrogen situations, unlobed leaves have significantly more centrally-located stomates than lobed leaves do. Where a Morus alba plant suffers severe nitrogen deprivation, the reverse is true.

There is uniformity to be seen in the overall larger size measurements for lobed leaf stomates versus unlobed leaf stomates (Table 2-V). However, the differences are significant only in the sun plant.

There is considerable variation in the stomatal counts of the lobed leaf in sun and in shade and with and without nitrogen. From this variation one could fairly draw the conclusion that a lobed leaf is a volatile unit, clearly responsive to situation. Coupling this fact with the statistically greater P_{net} production by lobed leaves on a droughted Morus alba, and statistically greater correlation of lobed leaves with incident PAR , a possible rationale for lobing begins to take shape. Lobed leaves may have greater phenotypic plasticity than unlobed leaves and this may be advantageous in stress.

A larger and more definitive study including more leaves (trees) and stomatal measurements may provide answers to this question of phenotypic plasticity in lobed and unlobed leaves in Morus alba.

Fig. 2- 1. Stylized lobed and an unlobed leaves showing areas from which sections of leaf used in the stomatal count were taken.

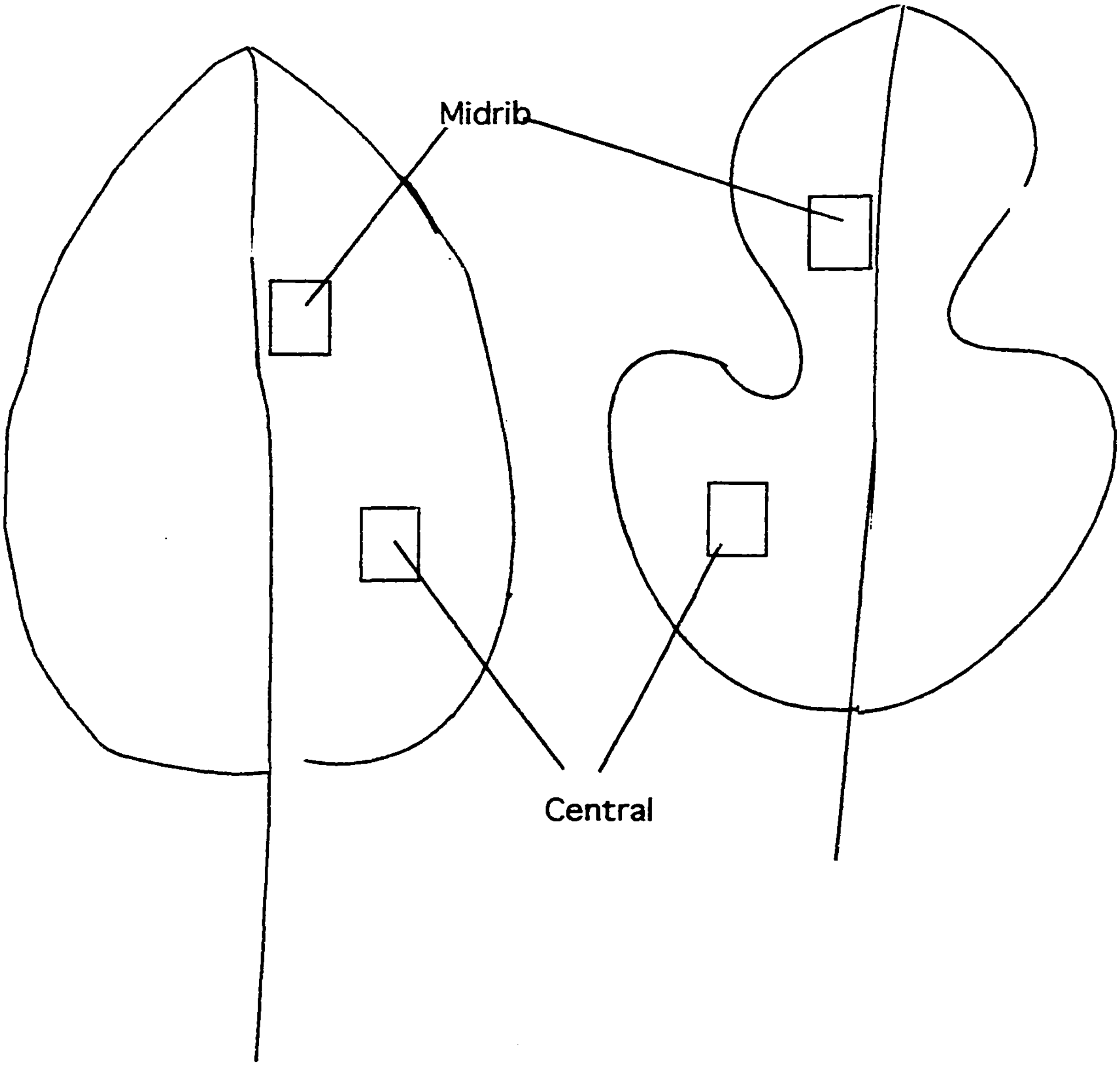
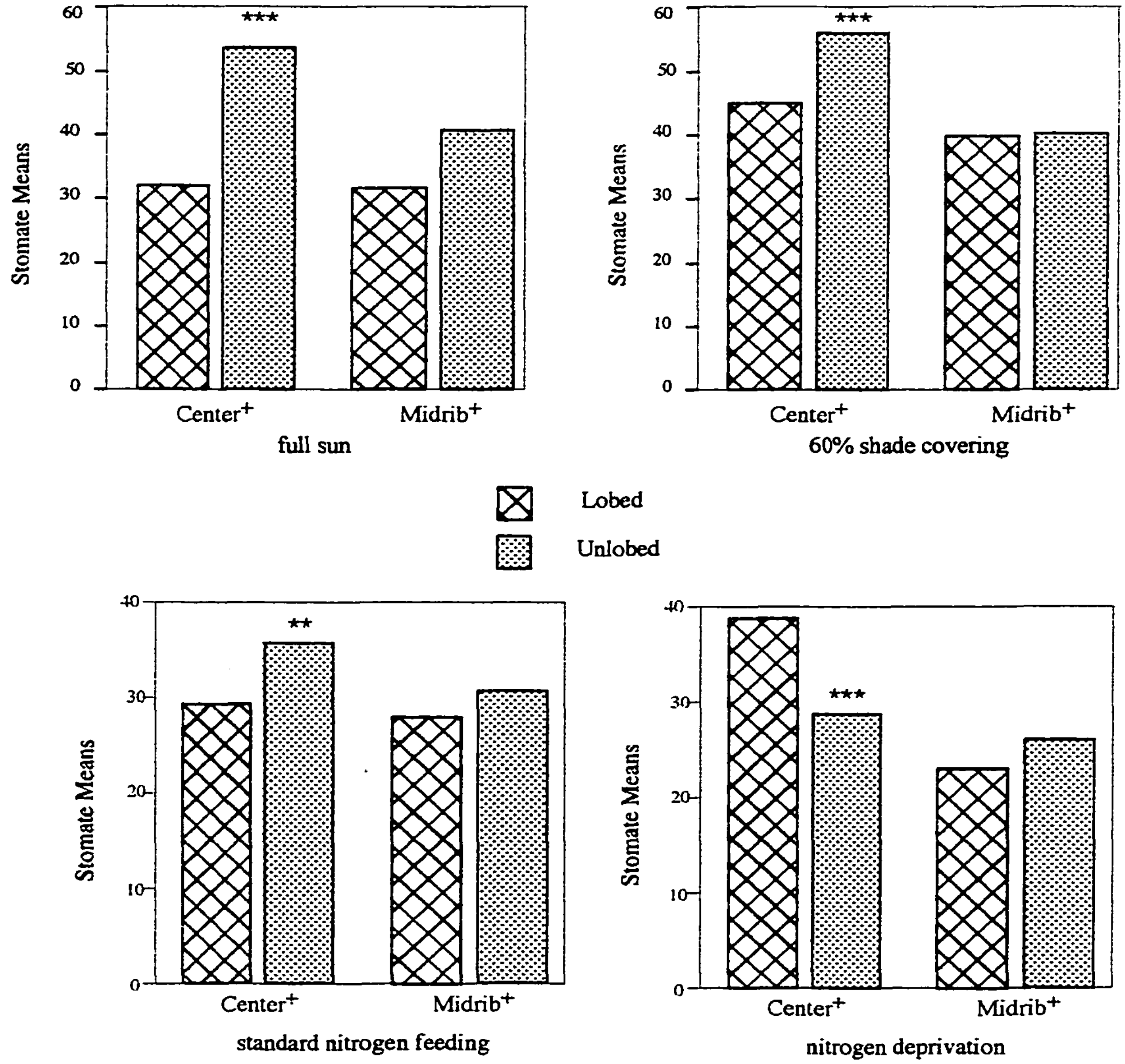


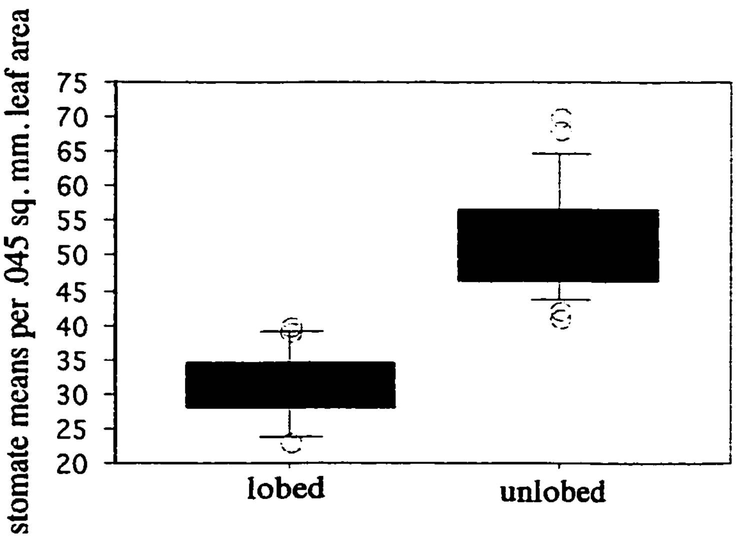
Figure 2-2. Bar graphs of ANOVA comparisons of mean stomate density of Morus alba leaves in four treatments. Asterisks above bars indicate statistically significant differences between lobed and unlobed leaves. P<0.01=*; P<0.001=**; P<).0001=***. N = 40 for each bar.



Center+ - in central portion of lamina

Midrib+ - near to midrib of lamina

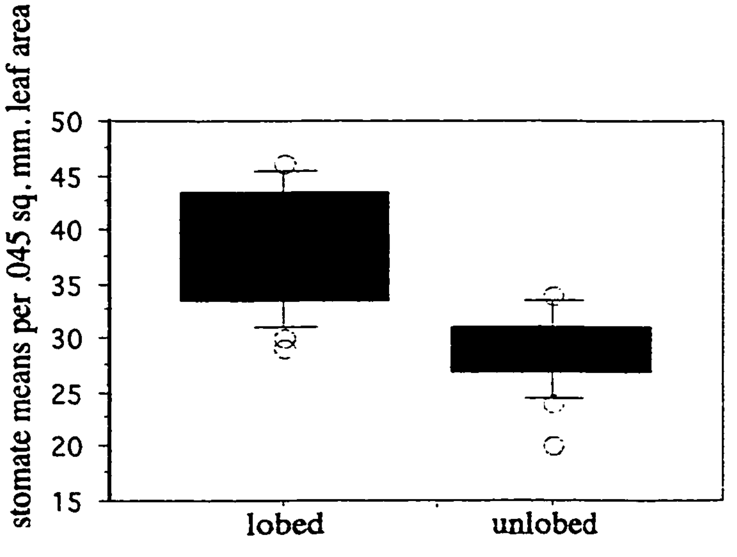
Figure 2-3. Box plots showing reversal of trend in stomate density for lobed and unlobed leavesin full sun (no stress) and with nitrogen deprivation (stress).



Full Sun

ANOVA Table for stomate means

	DF	Sum of Squares	Mean Square	F	P	$Power_{0.05}$
lobing cond.	1	4601.025	4601.025	106.938	<.0001	1.000
Residual	38	1634.950	43.025			



No Nitrogen

ANOVA Table for stomate means

	DF	Sum of Squares	Mean Square	F	P	$Power_{0.05}$
lobing cond.	1	1040.400	1040.400	49.667	<.0001	1.000
Residual	38	796.000	20.947			

Figure 2 - 4. Stylized stomate with indications of length and width of guard cells and length and width of aperture.

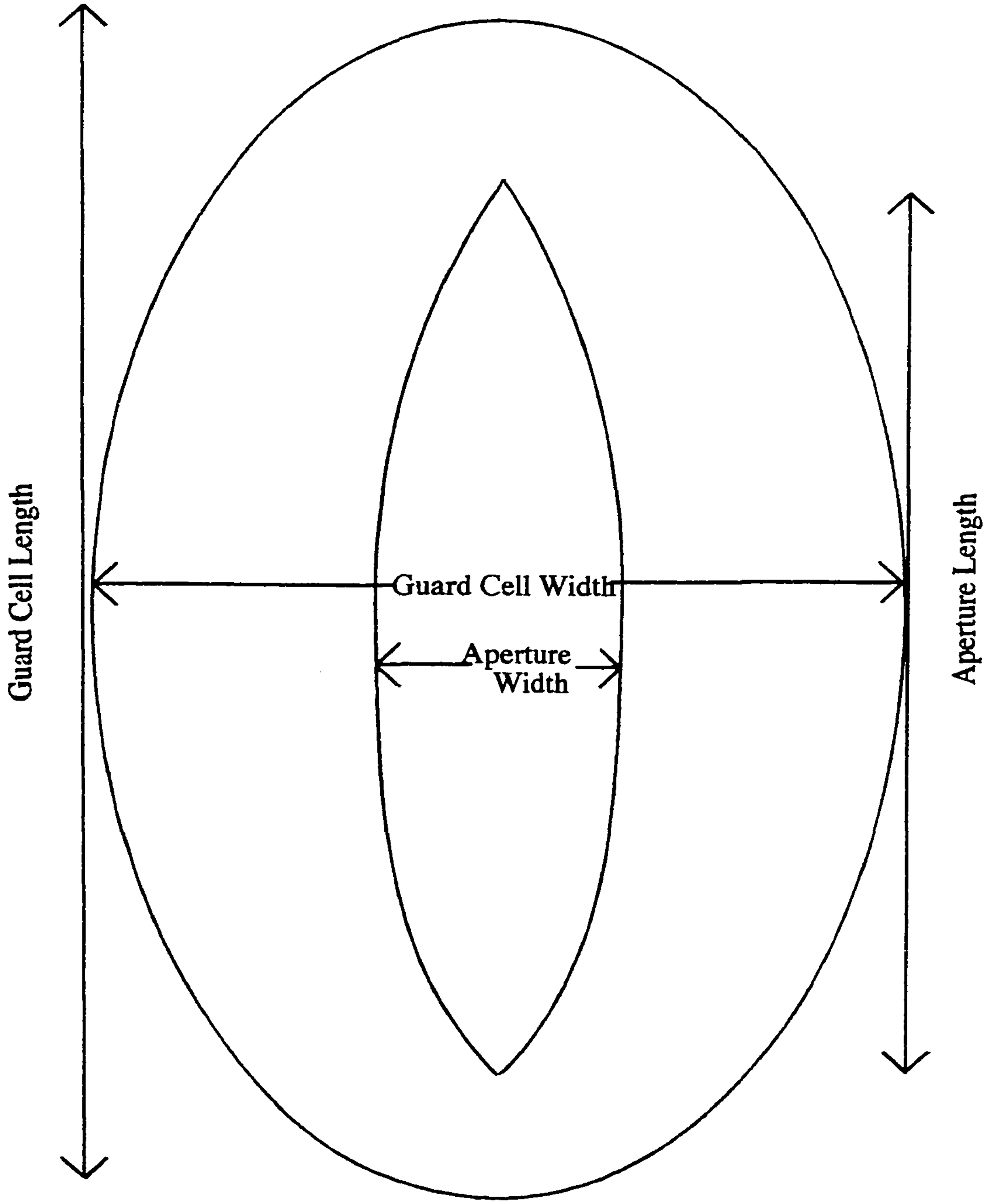
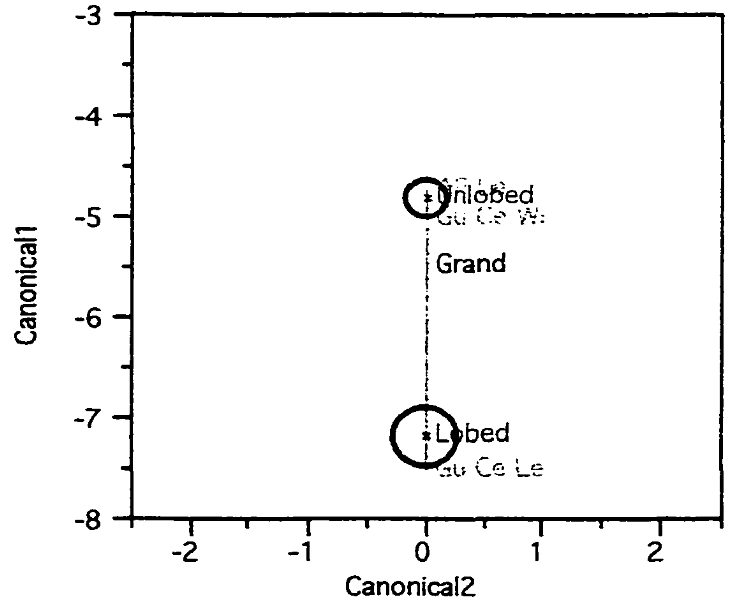


Figure 2 - 5. Canonical centroid plots generated in MANOVA for stomate measurements for lobed and unlobed leaves in sun and in shade.

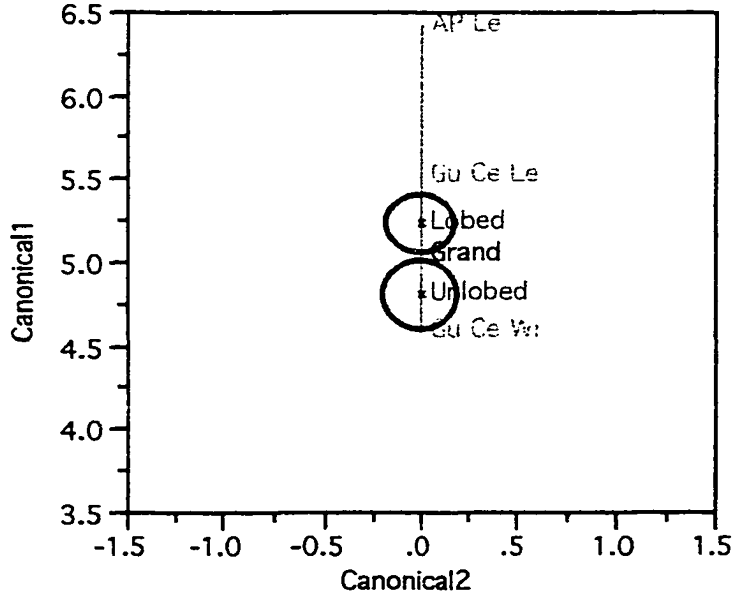
	F	v1	v2	P
Wilks' Lambda	60.9413	3	157	<.0001



Lobed/Unlobed

SUN

	F	v1	v2	P
Wilks' Lambda	3.2017	3	214	0.0242

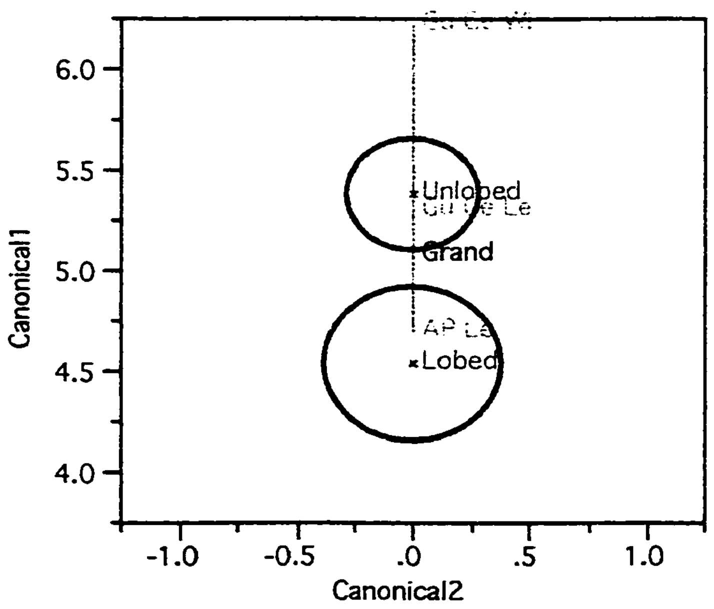


Lobed/Unlobed

SHADE

Figure 2- 6. Canonical centroid pots generated in MANOVA for stomate measurements of lobed and unlobed leaves with adequate nitrogen or nitrogen deprivation.

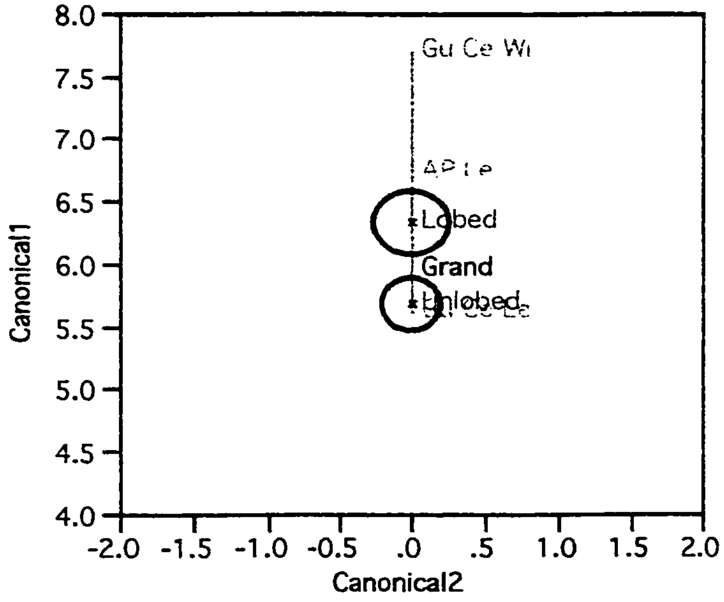
	F	v1	v2	P
Wilks' Lambda	3.9990	3	72	0.0108



Lobed/Unlobed

Nitrogen

	F	v1	v2	P
Wilks' Lambda	4.7728	3	143	0.0034



Lobed/Unlobed

Nitrogen deprived

Figure 2 – 7. Micrograph of centrally-located stomates in a shade lobed leaf. Area represents 0.045 sq. mm. of leaf magnified by 733.

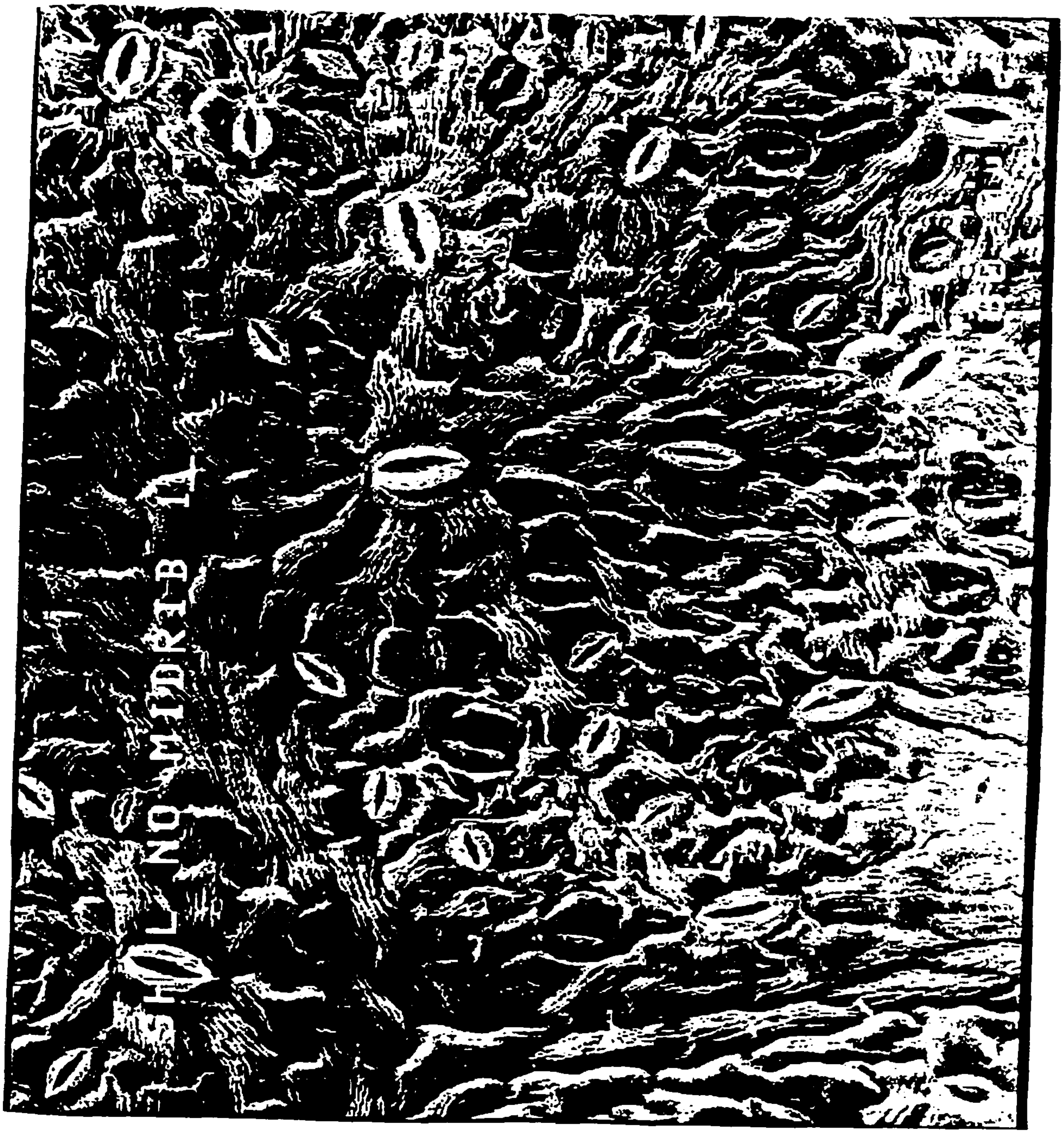


Figure 2 – 8. Micrograph of centrally-located stomates in a sun lobed leaf. Area represents 0.045 sq. mm. of leaf magnified by 733.

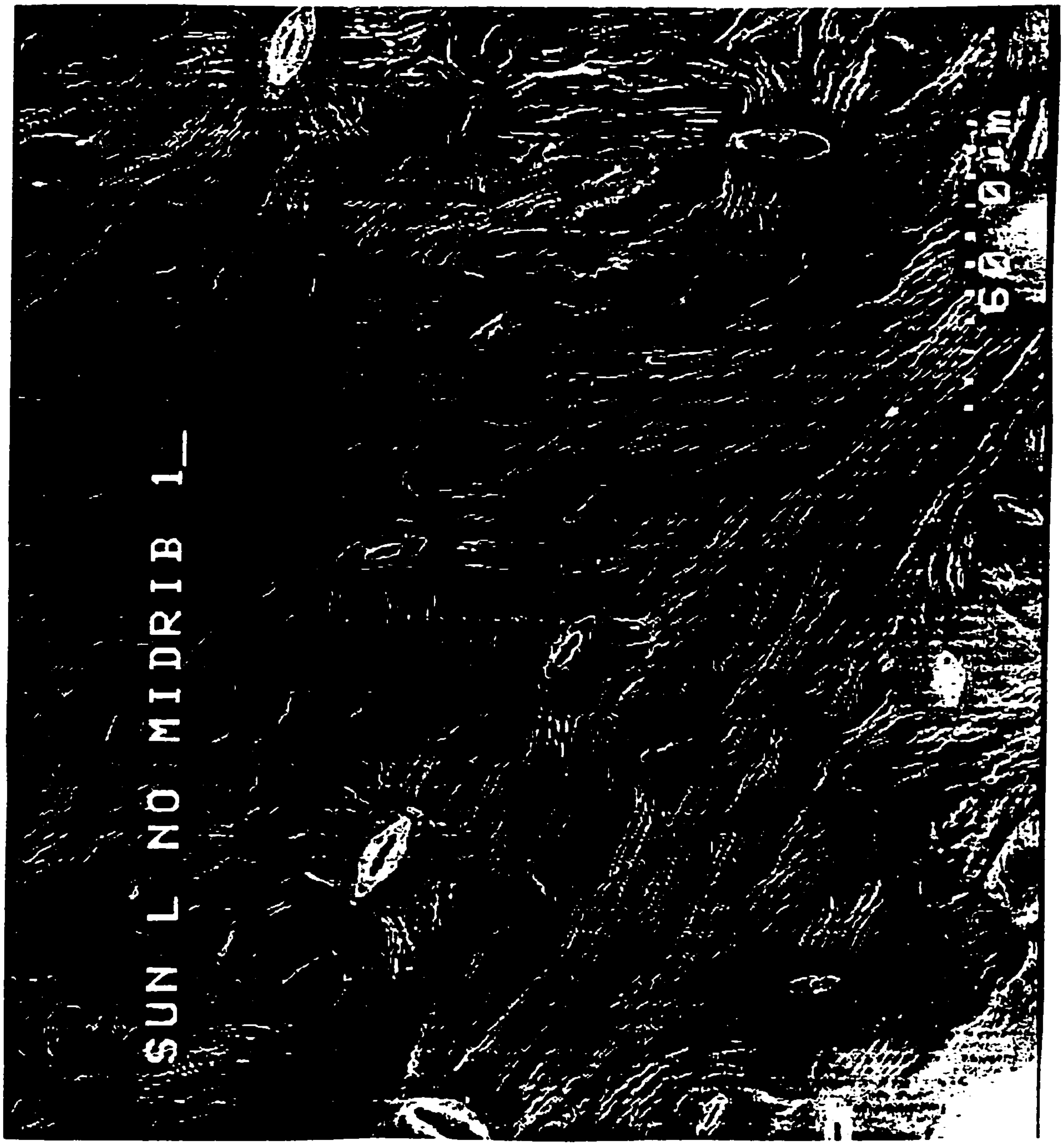
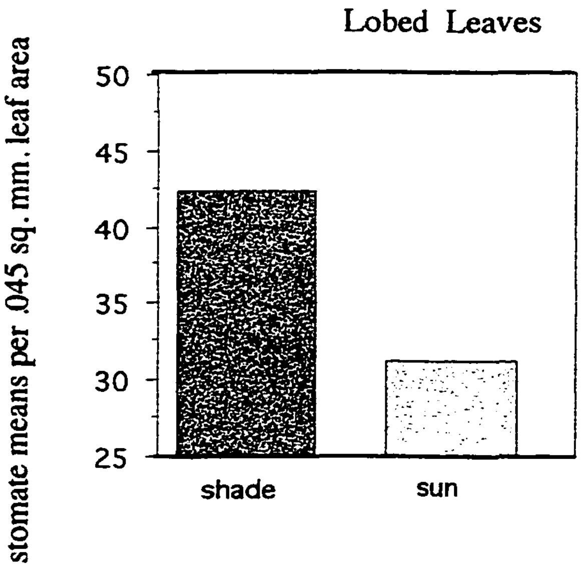
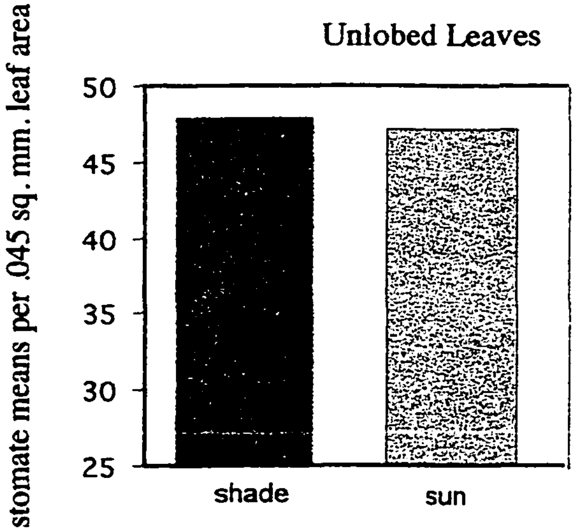


Figure 2- 9. Bar graphs of ANOVA results of mean stomate density of Morus alba leaves. Comparison is of lobed leaves in sun vs. shade, and of unlobed leaves in sun vs. shade.


Lobed Leaves

F	P	Rsq.
118.15	<0.0001	0.60

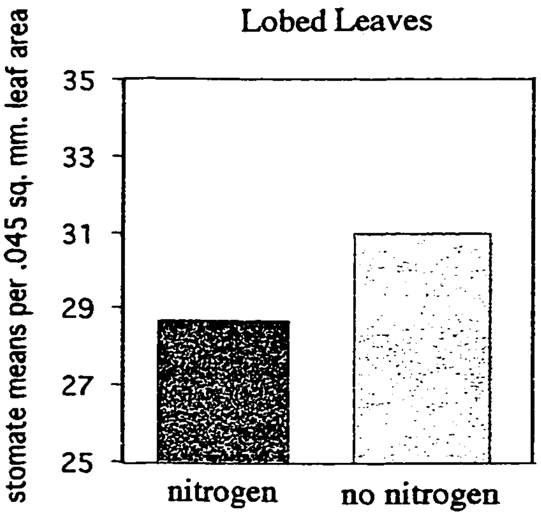
	Count	Mean	Std. Dev.
shade	40	42.35	4.43
sun	40	31.17	4.75


Unlobed Leaves

F	P	Rsq.
118.15	0.6274	0.003

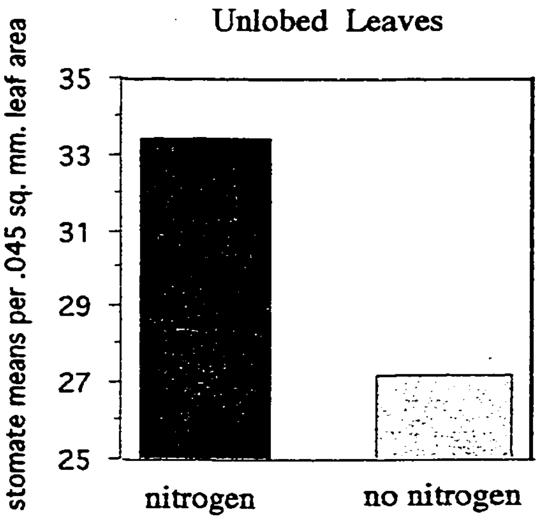
	Count	Mean	Std. Dev.
shade	40	47.88	10.01
sun	40	46.75	10.63

Figure 2- 10. Bar graphs of ANOVA results of mean stomate density of Morus alba leaves. Comparison is of lobed leaves with nitrogen vs. no nitrogen, and of unlobed leaves with nitrogen vs. no nitrogen.



F	P	Rsq.
1.54	0.2189	0.02

	Count	Mean	Std. Dev.
nitrogen	40	28.70	6.55
no nitrogen	40	30.95	9.43



F	P	Rsq.
23.18	<0.0001	0.23

	Count	Mean	Std. Dev.
nitrogen	40	33.38	6.80
no nitrogen	40	27.18	4.48

CHAPTER 3

LEAF CENSUS

SUMMARY

Five hundred leaves from 50 New York City and Dutchess county Morus alba trees were examined over the course of the growing season in 1996. An equal number of lobed and unlobed leaves from sun and shade trees were scored. Twelve variables were measured per leaf: internodal circumference, internodal distance, chlorophyll content, leaf specific mass, petiole length, blade length, blade width, perimeter, blade area, leaf complexity, dissection index, relative nodal position.

Significant differences were found between lobed and unlobed leaves in the overall sample in internodal distance, chlorophyll content, blade length, area, perimeter, leaf complexity, and leaf dissection.

Separate analyses were made of primary and secondary shoot leaves, and of sun and shade leaves in these situations. For secondary shoot leaves, no significant differences between lobed and unlobed leaves were noted.

Primary shoot leaves comprised the dominant component of the sample. Among these, there were multiple significant differences between lobed and unlobed leaves in both sun and shade; shade leaves registered the most differences. A lobed shade leaf was greener, had a shorter blade and petiole, more perimeter and greater dissection index, a thicker subtending shoot, a shorter distance to the next leaf, and was more proximally located on the shoot than an unlobed shade leaf.

Introduction

In the 1996 growing season, a total of 500 Morus alba leaves were examined in situ and then harvested for further analysis. Site information ghincluded nodal position of the leaf, total nodes of the shoot, whether the shoot was primary or secondary, nodal circumference below the leaf insertion point, chlorophyll content, sun or shade location, internodal distance to the leaf below. Lab analysis of each leaf included measurements of leaf specific mass, petiole length, blade length, blade width, area of the leaf, perimeter of the leaf, leaf dissection index, and leaf complexity index.

Materials and methods

Ten leaves, five lobed and five unlobed, were examined from each of 50 Morus alba trees in various situations (park, street, yard, highway verge, etc.) in Manhattan and the Bronx. The trees included both mature well-established specimens in full sun and smaller shade specimens. The only requirement for a tree to qualify for inclusion in the census was the presence and accessibility of at least five lobed and five unlobed leaves in good condition. A Minolta leaf greenness/chlorophyll meter (SPAD 501) was used to determine the chlorophyll content of each leaf nondestructively (Marquard and Tipton, 1987). Five measurements were made per leaf, avoiding major veins. The leaf specific mass (a combination of density and thickness, dry weight/surface area of one side of leaf (Witkowski and Lamont, 1991)) of each leaf was ascertained by removing ten six mm diameter discs, drying them at 70 degrees centigrade, and weighing them to the fourth decimal place. Blade width was measured at the widest point on the leaf. Blade length did not include the petiole. Area, perimeter, leaf dissection index and leaf complexity index were determined by digitizing the margin of each leaf according to the method of Kincaid and Schneider (1983). All tables and figures in this chapter were generated from the file entitled "pri. only 444 rows."

Results

The original sample of 500 leaves included primary and secondary shoots. Primary shoots are the latest year's growth. Secondary shoots grow out from primary shoots in the same season. The sample from the primary shoots (N=444) is much greater than that of the secondary shoots (N=56) as primary shoots were more abundant than secondary shoots. Preliminary analyses of secondary leaves indicated absolutely no differences between lobed and unlobed leaves on any level; accordingly, the investigation measured primary leaves in separate sun and shade situations. Descriptive statistics for the primary leaf groups were generated in all of the11 areas measured (Table 3 - I). In Fig. 3 – 9 the means for LSM, surface area, and chlorophyll content are graphed to show the difference between lobed/unlobed leaves and between shade and sun leaves. Included in Table 3 - I is a twelfth measure of variability between lobed and unlobed leaves created by assigning a relative nodal position score (nodal position/total nodes) to each leaf.

Table 3 - I. Mean, standard deviation, and count for 12 variables for lobed and unlobed sun and shade leaves from primary branches with P-value from one-way ANOVA. (ANOVA details in Table 3 - II). Internodal circumference to nearest mm., internodal distance, petiole length, blade length, blade width tonearest cm., perimeter to nearest mm., blade area to nearest sq.mm, LSM ($\mu g\ mm^{-2}$).

	Unlobed Shade			Lobed Shade			
	Mean	Std. Dev.	Count	Mean	Std. Dev.	Count	P
Internodal circumfer.	15.8	0.9	87	16.3	0.9	84	0.0008
Internodal distance	4.3	1.2	53	3.3	1.1	50	0.0001
spad chlorophyll	44.3	4.1	89	46.2	4.5	85	0.0036
lsm	92.48	26.74	89	95.4	20	85	0.42
Petiole length	4.8	1.2	89	4.1	1.5	85	0.0035
blade length	12.4	2.4	89	11.2	2.3	84	0.0001
blade width	9.3	1.8	89	9.3	1.8	82	0.92
perimeter	469.6	90.4	87	607.3	134.8	85	0.0001
blade area	8681	3034	87	6665	2430	85	0.0001
leaf complexity	3.4	1.6	87	10.3	2.1	85	0.0001
dissection index	1.5	0.15	87	2.1	0.28	85	0.0001
rel. nodal position	0.776	0.187	89	0.593	0.204	85	0.0001

	Unlobed Sun			Lobed Sun			
	Mean	Std. Dev.	Count	Mean	Std. Dev.	Count	P
Internodal circumfer.	16.6	1.3	137	16.8	1.6	127	0.25
Internodal distance	3.8	0.9	72	3.4	1.8	64	0.07
spad chlorophyll	44.9	4.2	139	46	4.4	129	0.03
lsm	135.2	41.1	139	132	37.9	129	0.51
Petiole length	4.4	1.1	139	4.5	1.4	129	0.41
blade length	12.9	2.1	139	12.1	2.4	129	0.003
blade width	9.5	1.9	139	9.9	2.0	127	0.03
perimeter	469.3	83.6	139	649.5	145.5	129	0.0001
blade area	9221	3190	139	7692	2718	129	0.0001
leaf complexity	3.2	0.8	139	10.5	2.2	129	0.0001
dissection index	1.4	0.09	139	2.1	0.29	129	0.0001
rel. nodal position	0.698	0.196	139	0.627	0.205	129	0.004