SOME MORPHOLOGICAL, ECOLOGICAL, AND PHYSIOLOGICAL TRAITS OF FOUR OZARK FOREST SPECIES [1/]

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ABSTRACT

Studies were conducted on forest grown saplings of sugar maple (Acer saccharum Marsh), northern red oak (Quercus rubra L.), white oak (Q. alba L.) and black oak (Q. velutina Lam.) on three different sites in mid-Missouri. Eco-physiological measures included xylem pressure potential and leaf conductance while the morphological factors observed were the length of the stomatal pore and stomatal density. Decreasing soil moisture had the greatest effect on leaf conductance in sugar maple and the least on that in black oak. Northern red oak and white oak were intermediate in response. Species' differences in leaf conductance and xylem pressure potential were more pronounced on a xeric than on a mesic site. Stomatal morphology data did not suggest any consistent differences in stomatal density or pore length which would account for the observed eco-physiological differences.

INTRODUCTION

The interaction between environment, plant morphology and plant function has been of interest to scientists for many years. Goebel in 1900 wrote in his Organography of Plants (cited by Foster and Gifford 1959) "the form and function of an organ stand in the most intimate relation to each other." Stomata have ultimate control over water movement

[1/] Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 7542.

through the plant since stomatal conductance controls the rate of evaporation from the leaves (Jarvis 1975). Stomatal conductance in turn is affected by several environmental and plant factors as they alter guard cell turgor.

Morphological, physiological, and hence ecological adaptations which allow a plant to maximize carbon dioxide uptake for growth, while being able to avoid drought and/or heat stress, exist to varying degrees in all species. For example, variable leaf structure as it affects energy exchange has been discussed in detail by Gates (1968). However, the ecological importance of variable stomatal frequency and size coupled with differing stomatal reactivities to water stress and light has rarely been discussed (Salisbury 1927; Carpenter and Smith 1975).

The purpose of this paper is to (1) present diurnal patterns of stomatal conductance and xylem pressure potential for widely divergent soil moisture levels, (2) describe stomatal reactivity to light under high and low soil moisture conditions and (3) characterize stomatal density and stomatal pore length in four deciduous species common to the oak-hickory forest type.

MATERIALS AND METHODS

Studies were conducted within a second growth oak-hickory forest at the Ashland Wildlife Area 9 km east of Ashland, Missouri in southeastern Boone County. The climate of the area is typical of the humid continental type with an annual average temperature of 13°C and total precipitation averaging 941 mm per year.

Plant Material

Two understory saplings (2.5 - 3.5 m tall and 1.0 - 5.0 cm diameter at 1.3 m) of each species, white oak (*Quercus alba* L.), northern red oak (*Q. rubra* Ehrh.), black oak (*Q. velutina* Lam.) and sugar maple (*Acer saccharum* Marsh.), were selected on each of three 400 m^2 plots. These plots were chosen to insure a wide range of soil and microclimatic conditions. The xeric plot was located on a steep south-facing slope (26%) with a shallow rocky soil of high clay content. The old field plot occupied a ridge top covered with a deep loess cap. The forests on these plots were characterized by relatively open canopies. The mesic plot occupied an upper slope position in a south facing drainage (12% slope) with an eroded soil of high silt content and had a well developed forest and a closed canopy. This plot was protected by ridges on the east and west sides.

Stomatal Reactivity Procedures

Data were collected sequentially on 42 days beginning on the xeric plot on day 1, then the old field plot on day 3, the mesic plot on day 5 and so on. On each day from 0500 until 2000 hours (true solar time), the following sampling scheme was followed. Photosynthetically active radiation (PAR) incident upon the leaf and abaxial leaf surface resistance were measured at hourly intervals on two leaves from each sapling of each species. Xylem pressure potential (P) was taken on petioles of the aforementioned leaves at 0500, 0900, 1200, 1500 and 1900 hours.

Leaf surface resistance was measured on the abaxial side of the leaf using a Lambda Model LI-60 Diffusive Resistance Meter and a Model LI-15S horizontal sensor similar to that described by Kanemasu et al. (1969). The procedures and precautions outlined by Morrow and Slatyer (1971) were followed. Leaf conductance was calculated by taking the inverse of leaf resistance. Photosynthetically active radiation (PAR: 400 - 700 nm) was measured at the leaf surface using a Lambda LI-190S Quantum Sensor (Biggs et al. 1971). Following procedures outlined by Ritchie and Hinckley (1975), a pressure chamber was used to estimate xylem pressure potential (P).

Soil moisture was measured at least twice a week using a Nuclear Chicago Model P-19 depth moisture probe and a Nuclear Chicago 2800-A scaler. Soil moisture measured with the nuclear probe was calibrated against soil moisture tension values measured with a Soil Moisture Equipment Company 1200 Pressure Plate extractor and 1000 Pressure Membrane extractor for each 15 cm layer of each soil profile.

Stomatal Morphology Procedures

Several leaves were removed from the mid-crown of understory saplings of each species and placed in sealed moisture chambers for transportation back to the University of Missouri. Samples from 5 to 8 mm^2 were cut from each leaf at a point midway between the midvein and the margin. Necrotic areas and areas containing large lateral veins were avoided. Immediately after the samples were cut, they were placed in 2% osmium tetroxide (in KH_2PO_4 buffer) and allowed to fix overnight. The following day the samples were washed in buffer, then in distilled water, and dehydrated in a graded series of ethyl alcohol ranging up to 100%, transferred through a graded series of isoamyl acetate to 100% and critical point dried according to a modification of the precedure of Anderson (1951). Samples were mounted on specimen stubs, coated with gold and scanned at a 45 degree tilt and 10 kV.

Stomatal pore length (length of the throat) and density were computed from 89 mm x 114 mm micrographs taken directly from the scanning electron microscope. Values were adjusted to compensate for the 45 degree tilt of the specimen stage. Stomatal pore lengths were determined using micrographs taken at 1000 X magnification; while frequency data were

obtained from micrographs taken at 300 X magnification. In order to reduce bias and to obtain an accurate count of density, stomatal density data were obtained from randomly selected areas within each leaf sample. Three leaves and four sub-samples from each leaf were examined for each species.

RESULTS AND DISCUSSION

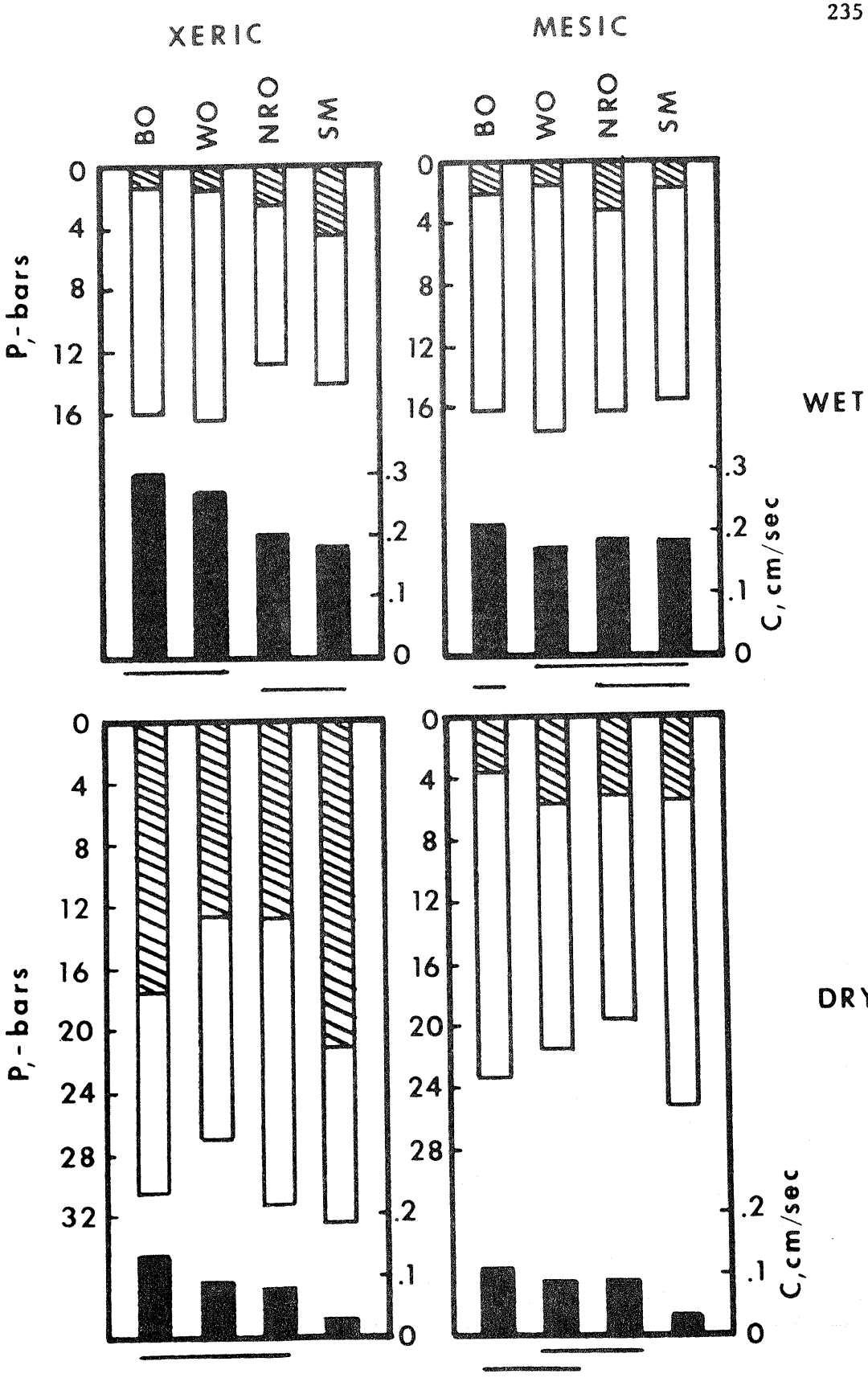
The summer of 1974 was characterized by a progressive drought which began on June 20 (day 171) and lasted until August 2 (day 214). During this 43 day period, 2.8 cm of precipitation fell and such species as sunflower (Helianthus strumosus L.) and flowering dogwood (Cornus florida L.) were noted to be continuously wilting. Recovery from this prolonged drought was initiated in early August; however, soil moisture did not return to pre-drought levels until early September. Eco-physiological results were divided into two sections: (1) analysis of diurnal patterns using selected days on the two plots before and at the peak of the drought and (2) analysis of light and xylem pressure potential effects on stomatal reactivity using all study days.

Diurnal Patterns

Data from four warm, clear days are presented for a comparison of the extreme soil moisture conditions found during this study (Fig 1). Day 174 (June 23, xeric plot) and day 180 (June 29, mesic plot) were characterized by soil moisture levels (average for upper 45 cm) near field capacity (-0.8 and -0.4 bars, respectively). Soil moisture on the dry days was considerably less than field capacity and was -10.5 bars on the mesic plot on day 208 (July 27) and -11.2 bars on the xeric plot for day 210. Base P (xylem pressure potential at 0500 hours) ranged from a maximum of -1.3 bars to a minimum of -21.2 bars and varied by day, site and species.

On the xeric plot under conditions of high soil moisture, black oak and white oak maintained higher average leaf conductance than the more mesic species, northern red oak and sugar maple (Fig 1). When light levels were above saturation for stomatal opening, highest average conductance values were observed in black oak throughout the day. The more moderate microclimate of the mesic plot may in part explain the differences in black oak and white oak leaf conductance values for these two sites. Light intensity was generally higher at sapling levels on the xeric than on the mesic plot. Base P values, an equilibrium between plant and soil water potential (Ritchie and Hinckley 1975), were approximately the same on both plots and in all species except sugar maple on the xeric plot. These lower base P values in sugar maple suggested shallow rooting behavior in sugar maple as compared to oak.

Figure 1. Diurnal patterns of base P (hashed bars) average daily P (open bars) and leaf conductance (C-solid bars) for a xeric and a mesic site on a day of high soil moisture (wet) and a day of low soil moisture (dry). Lines on common levels below each graph indicate no significant differences at the 0.05 level.


XERIC
MESIC
BO
WO
NRO
SM
P, - bars
C, cm/sec
WET
DRY

Under conditions of relatively low soil moisture, leaf conductances in oak on the mesic plot were consistently higher than those of sugar maple (Fig 1). Xylem pressure potential was also lower in sugar maple than in the other three species. These data indicated that even on a relatively sheltered site with soil moisture deficits, the effects of plant water deficits were more apparent in sugar maple than in oak. Though the average decrease in both base P (10.9 bars) and average P (7.9 bars) was substantial when comparing the species on the xeric plot to those on the mesic plot under dry soil conditions, leaf conductance values did not change appreciably. Leaf conductance remained approximately the same in northern red oak, white oak and sugar maple when comparing the two plots; however, it actually was greater (stomata more open) in black oak. Data from these four days suggested that leaf conductance in these four species was possibly affected differently by changing soil moisture and decreasing xylem pressure potential.

Stomatal Reactivity: Stomatal Response to P

Hydroactive stomatal closure occurs in plants when water deficits exceed a critical value (Turner 1974). If stomatal closure was initiated at an identical xylem pressure potential in all species, then closure and hence reductions in evaporative cooling would be noted sooner in species on xeric sites than in those on mesic sites. The critical xylem pressure potential which leads to a decrease in leaf conductance was determined for each species in this study. This critical potential was -17.0 bars in sugar maple, -18.5 bars in northern red oak, -23.0 bars in white oak and -24.5 bars in black oak. Thus, when comparing these four species, higher leaf conductances were maintained at greater water deficits in xeric than in mesic species. Examination of diurnal patterns suggested that sugar maple reached critical P levels sooner on both a diurnal and seasonal basis than the other species. In contrast, black oak was able to maintain open stomata longer under these same conditions.

Stomatal Reactivity: Stomatal Response to Light and Soil Moisture

Statistical analyses were performed on leaf conductance and PAR values in each species for periods when soil moisture was high (base P > -3.0 bars) and for periods when soil moisture was low (base P < -3.0 bars). The least squares best fit equations were then used to determine the leaf conductance and PAR values presented in table 1. Leaf conductance when PAR equals zero provided an estimate of the minimum or cuticular conductance. Under conditions of high soil moisture, this minimum value decreased from sugar maple to northern red oak to white oak and finally to black oak. As the soil dried, minimum conductances tended to equate amongst these species. Significantly greater decreases in leaf conductance were noted in sugar maple than in black oak as the soil dried. Ranking these species according to shade tolerance, one might have the following order: sugar maple > northern red oak > white oak > black oak. In contrast, a possible drought tolerance ranking might be: black oak > white oak = northern red oak > sugar maple. The previous discussion on diurnal patterns appears to support this last ranking.

Table 1. The relationship between leaf conductance and photosynthetically active radiation (PAR) is analyzed in sugar maple (SM), northern red oak (NRO), white oak (WO) and black oak (BO) under dry and moist soil moisture conditions. Only PAR values less than 300 $\mu E/m^2/sec$ were analyzed in order to eliminate confounding effects due to such factors as high leaf temperature and low xylem pressure potentials.

	Leaf Conductance When PAR = 0		Leaf Conductance When PAR = 300 $\mu E/m^2/sec$			PAR When C = 50% of Max.	
Species	Moist	Dry	Moist	Dry	Change	Moist	Dry
	---cm/sec----		---cm/sec----		--%---	---$\mu E/m^2/sec$--	
SM	.036	.019	.333	.128	62	16.9	27.0
NRO	.031	.020	.357	.238	33	20.8	26.2
WO	.028	.019	.435	.303	30	21.9	32.8
BO	.023	.019	.313	.263	16	25.8	34.6

Gates (1968) and Knoerr and Gay (1965) presented evidence that greater rates of transpiration and, therefore, more dense and/or larger stomata would be of an ecological advantage to xeric species. Results from the eco-physiological data presented herein suggested that there may be very different stomatal morphologies between these species especially between black oak and sugar maple. This possibility was investigated.

Stomatal Morphology

Adaxial and abaxial surfaces of the leaves of each species were examined and all leaves were found to be hypostomatous. Thus all measurements presented are from the abaxial (lower) surface of the leaves. Measurements of the external length of the longitudinal axis of the stomatal pore were found to be indicative of the size of the internal stomatal pore. This observation was made from views of transverse sections through leaf samples of each species. A statistical analysis was performed between each species for mean stomatal density and mean stomatal pore length (Table 2).

Maple leaves were characterized by protruding epidermal and guard cells (Fig 2). Stomata were randomly oriented and the pore length averaged 10.08 micrometers (μm), but this size varied considerably (Table 2). Stomatal density was found to be 351 stomata per mm^2 of leaf surface area.

Table 2. Stomatal density and stomatal pore length (with standard deviations) data. Note that parameters with the same superscript in each column are not significantly different from each other at the 0.05 level. Test used was a student's t-test.

	Stomatal Density/mm^2	Stomatal Pore Length (μm)
Sugar maple	351 ± 60^{a}	10.08 ± 1.22^{a}
Northern red oak	355 ± 58^{a}	11.33 ± 1.53^{b}
White oak	$302 \pm 31^{b,c}$	10.85 ± 1.66^{b}
Black oak	$325 \pm 71^{a,c}$	15.51 ± 1.40^{c}

The stomatal pores of red oak were somewhat larger than those of maple averaging 11.33 μm in length. Epidermal cells did not protrude as much, but guard cells did protrude (Fig 3). Pubescence was present on the surface but the hairs were few and widely spaced. Stomatal density was 355 per mm^2.

White oak guard cells (Fig 4) were observed to be narrower than those of the other species. The average stomatal pore length was the smallest of all the oaks (10.85 μm). White oak appeared to be more pubescent than either red oak or sugar maple and the pubescence seemed to be associated with the vascular tissues of the leaves. The epidermal cells of white oak protruded as did those of sugar maple. It also was observed that deposits (presumably waxy) occluded most of the stomata (Fig 5). The average stomatal density of white oak was lower than those of the other species studied (302 per mm^2).

Black oak had the largest stomatal pore size (15.51 μm) and the greatest amount of pubescence of the species studied. The surface of the leaf was somewhat smooth except for the pubescence and protuberences of the guard cells (Fig 6). Pubescence was observed to occur in either single strands or in tufts of eight hairs. Where hairs occurred, they appeared to be associated with vascular tissue. Stomata averaged 325 per mm^2, representing an intermediate density between white oak and northern red oak.

Early work by Salisbury (1927) using stomatal density data, indicated that density generally increased from mesic to xeric species. However, the relationship between increasing stomatal density and increasing xerophytic conditions was not evident in our findings nor in those of Carpenter and Smith (1975) when they examined leaves of northern red oak, white oak and black oak. In our study, the greatest difference was noted between stomatal pore length in sugar maple as compared to that in black oak.

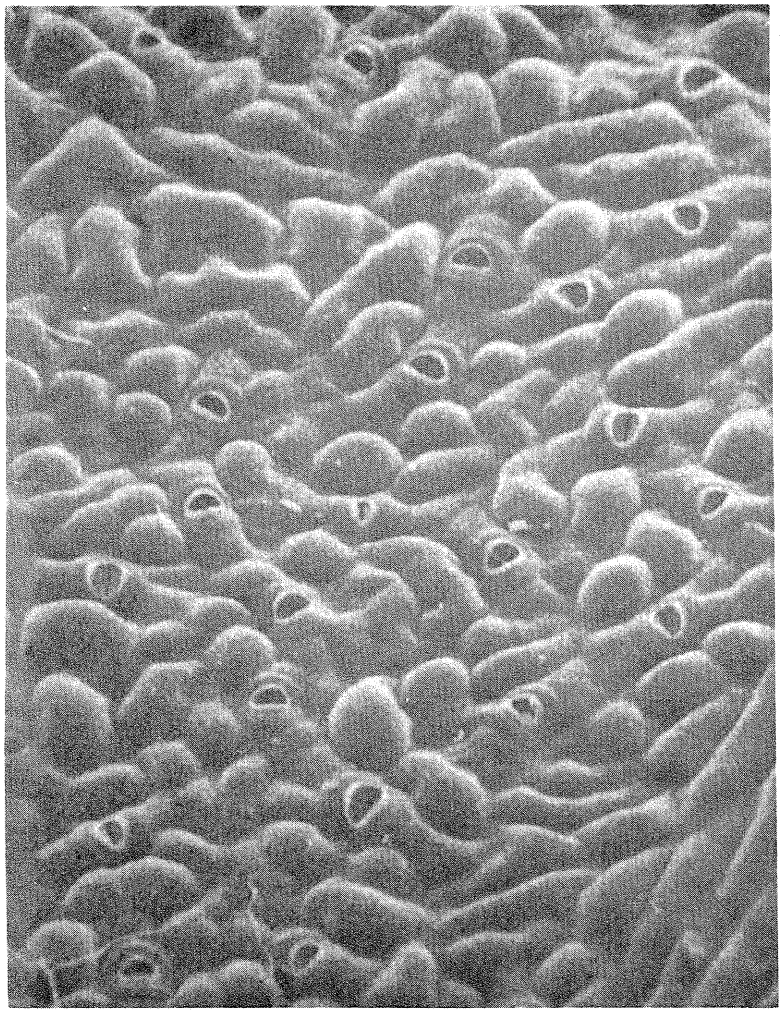


Figure 2. Micrograph of abaxial surface of maple leaf at 540X. In this and similar areas stomatal size and distribution measurements were taken. Note external topography of leaf.

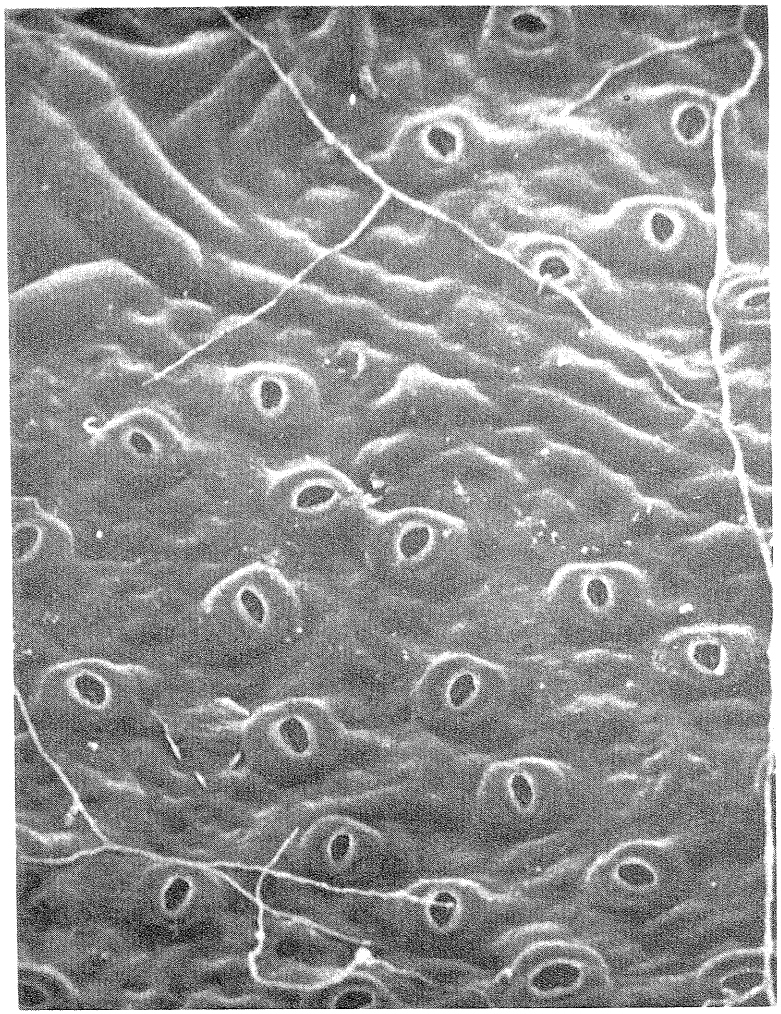


Figure 3. Micrograph of abaxial surface of red oak leaf at 540X. In this and similar areas stomatal size and distribution measurements were taken.

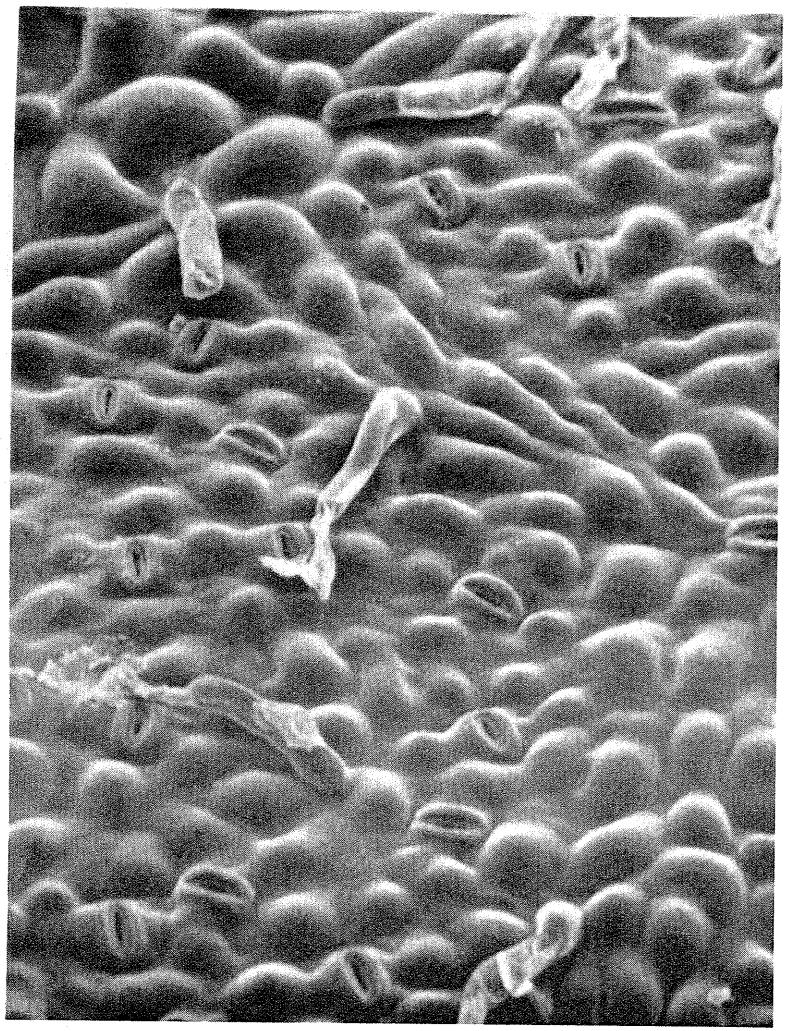


Figure 4. Micrograph of abaxial surface of white oak leaf at 540X. In this and similar areas stomatal size and distribution measurements were taken. Note different shape of stomata.

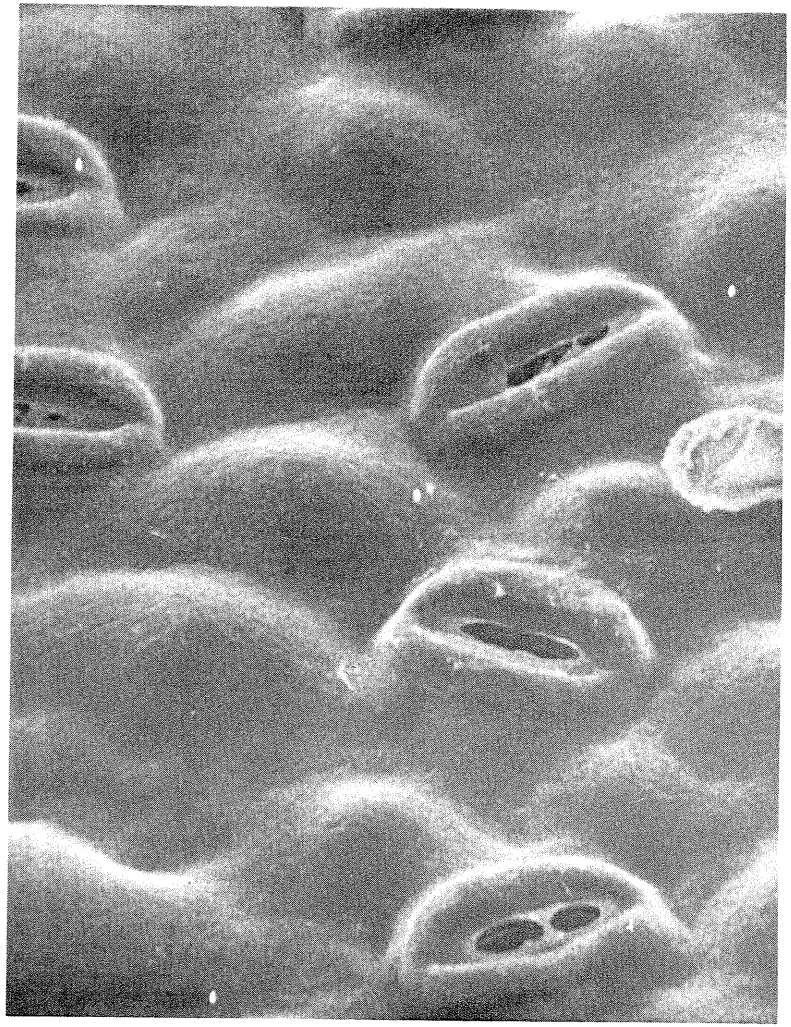


Figure 5. Micrograph of abaxial surface of white oak leaf at 1800X. Note waxy deposits in stomatal aperture.

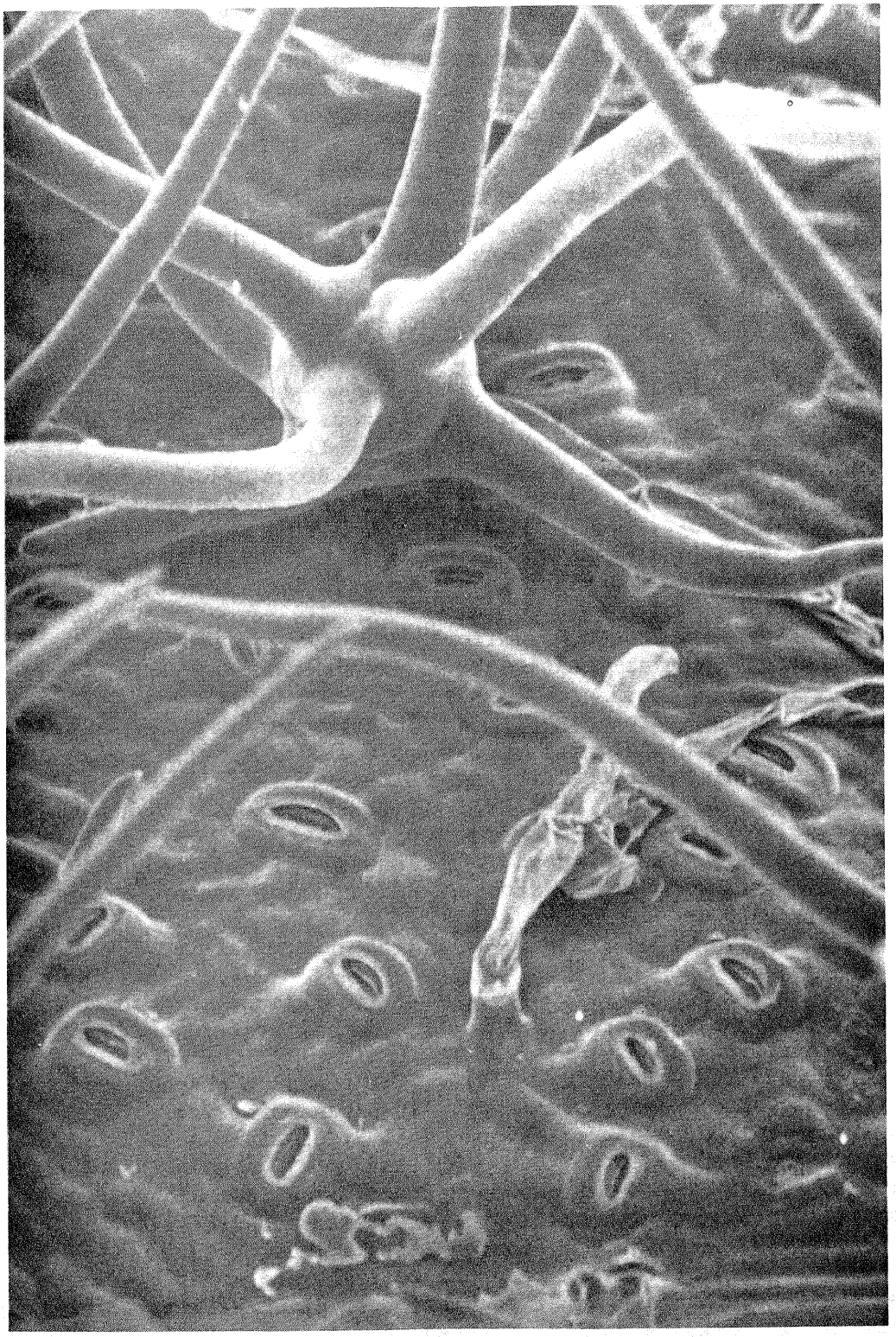


Figure 6. Micrograph of abaxial surface of black oak leaf at 540X. Note pubescence on leaf and external topography.

Other differences were either not significant or the opposite of those originally suggested by Salisbury (1927). In summary, small morphological differences such as observed herein may only be important under either optimum (maximum stomatal opening) or severely limiting conditions (minimum stomatal opening). In between these extremes, the stomatal mechanism as it reacts to different environmental and plant cues appears more important in determining how these four species react to changing plant and soil water deficits than stomatal morphology.

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ACKNOWLEDGMENTS

Thanks to Dr. Merton F. Brown for the use of E. M. facilities of the College of Agriculture.

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