Geographic Patterns of Variation in Biomass Production of California Blue Oak Seedlings as a Response to Water Availability[1]

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Abstract

This study characterized the distribution of geographic variation in water relations traits for six populations of California blue oak along a precipitation gradient (two each from high, medium and low precipitation environments). Acorns were collected from each population, germinated and the resulting seedlings were experimentally treated with three different watering regimes in a greenhouse experiment. There were significant among-population differences in growth and biomass production characteristics in the seedlings. Significant among-treatment differences indicated at least low levels of plasticity, although there were no treatment x population interactions. Implications are that seed source zone maps should be used by restorationists until we have more information that coadapted or regional gene complexes do not exist.

Introduction

California's blue oak (*Quercus douglasii*) populations are found in areas that have historically received high levels of precipitation, as well as in much more arid areas. This study examined whether there are differences in seedling morphology among blue oak populations that reflect the action of natural selection on traits that respond to water availability. In this study, blue oak acorns were collected from six populations, germinated and cultivated in a greenhouse for one growing season, and subjected to three different watering treatments. The resulting effects on growth and biomass production of seedlings from each of the populations in each treatment were assessed.

Populations were different in latitude, longitude, elevation and average amount of precipitation received annually *(table 1)*. Multiple regression of ecological characteristics with means of plant morphological traits was used to assess whether among-population variation reflected the precipitation gradient, which would suggest that the two are linked. This study also suggested that there were significant

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differences among these populations in traits whose means were significantly correlated with the water availability gradient in blue oak seedlings.

Table 1—*Locational and precipitation descriptions of the six blue oak populations included in the study.*

Pop	elevation (m)	latitude	longitude	30 yr precipitation normals (in)
1	325	40.40	122.25	60.80
5	150	40.18	122.06	28.07
6	725	39.26	121.16	66.17
16	600	37.59	120.22	31.55
23	150	37.02	119.37	13.77
25	800	35.42	118.47	18.73

California, because of the great variety of its climatic regimes and land forms, has more known biological diversity in its vascular plants, in terms of species richness, than any other state in the U.S. (Smith 1987). Many widespread species are not listed as threatened or endangered, but nonetheless, are losing the integrity of their gene pools, foreclosing evolutionary potential and resilience. Thoughtful planning could result in conservation of genetic diversity in these widespread species, preserving their evolutionary fitness by maintaining populations that have adaptations to local conditions. Knowledge of the factors that initiate and maintain patterns of variation are instrumental in establishing effective and efficient conservation practices (Hamrick and others 1991).

There is evidence that California's blue oak (*Quercus douglasii* H. & A.) populations are not regenerating at a rate that will maintain their current densities in certain areas of the state's oak woodland communities (Griffin 1971, Muick and Bartolome 1987). The apparent decline in the regeneration of blue oak has stimulated interest in conservation strategies for the species.

Information on geographic patterns of variation in blue oaks could be used to establish seed source zone maps. Establishing these maps could increase the long term success rate of planting and restoration projects by maintaining ecosystem integrity, since acorns from ecologically similar areas are more likely to produce blue oaks with necessary adaptations. Since blue oak is a keystone species, this may be important in the maintenance of ecosystem structure. In addition, delineation of seed collection zones will help maintain genetic integrity of populations and avoid the costs of rehabilitating the genetic diversity of a rare species, which can be prohibitive (Millar and Libby 1991).

Methodology

Maps of locations of six populations represented in common gardens established by McBride and others (1996) were overlaid with localities of California Cooperative Weather Stations. Statewide climatic data, stored at the National Climatic Data Center in Asheville, NC, were used to identify which of the fifteen Sierran populations represented in two common gardens have received high, middle, and low volumes of precipitation over a preceding 30 year period, based on weather information compiled between 1961 and 1991 by Owenby and Ezell for the National

Oceanic and Atmospheric Administration (NOAA 1992). Two populations from each of three precipitation patterns were selected (precipitation normals of approximately 60 inches, 30 inches, and 16 inches per year) (*fig. 1*).

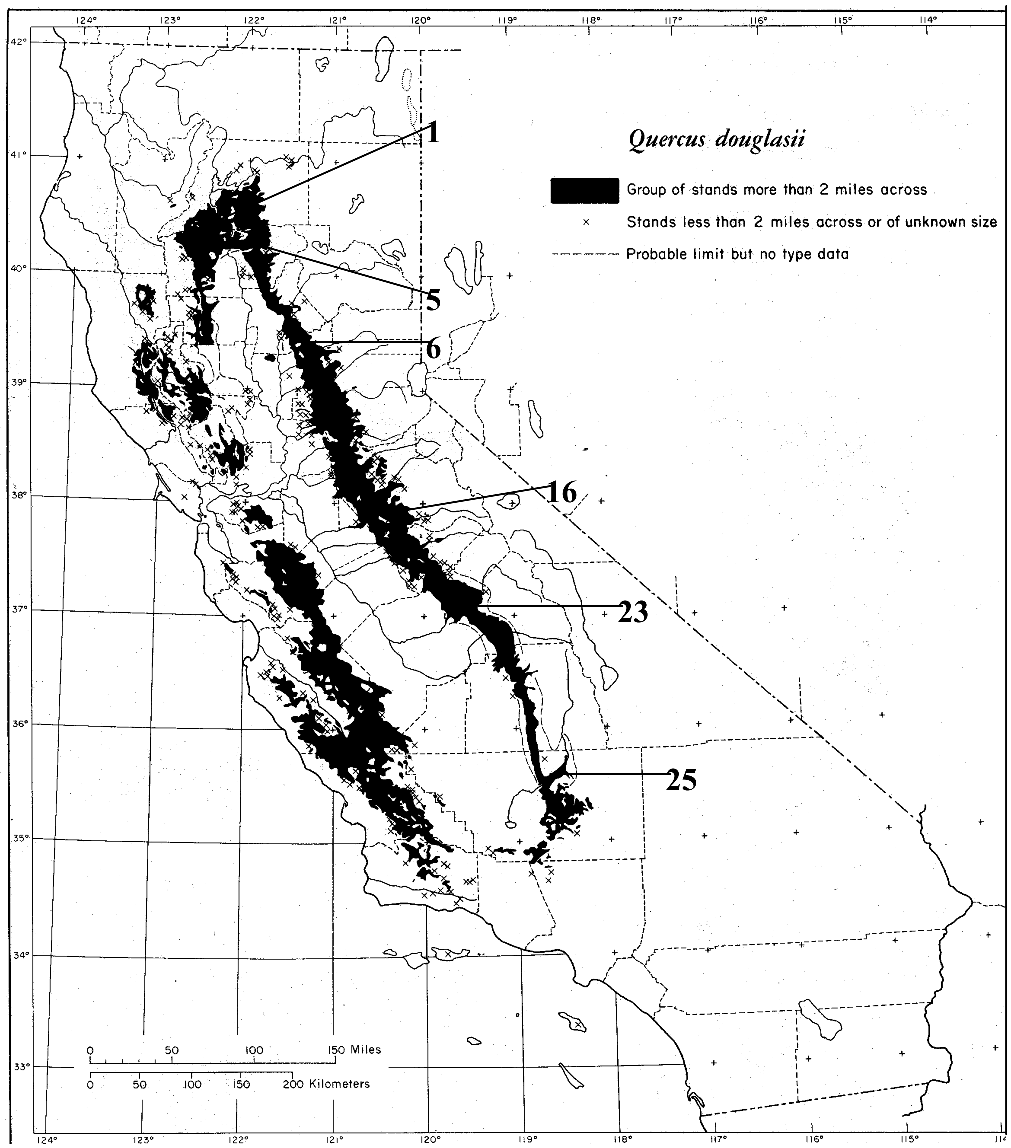

Figure 1—Map of blue oak populations included in this study, selected because of similarity and proximity to weather stations. (Adapted from Griffin and Critchfield 1972).

Acorns were collected from each of the six populations in October 1996. Ten trees, no closer than one hundred meters were selected in each population; approximately 100 acorns from each tree were gathered and bulked (McBride and others 1996). Acorns were dried to approximately 30 percent moisture content, placed on flats and refrigerated. Acorns remained in cold storage at approximately 36º F, until all populations reached 27 percent water content. The acorns were germinated in greenhouse flats in vermiculite. When most of the acorns from all populations had germinated, the germinated acorns were then randomly assigned and transplanted into individual containers.

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The acorns were germinated (emergence of the radicle) in greenhouse flats in vermiculite. Germination rate for all acorns was approximately 98 percent. As acorns germinated, they were returned to cold storage to inhibit growth until they were planted. When most of the acorns from all populations had germinated, the germinated acorns were then randomly selected, assigned and transplanted in pairs into individual containers similar in construction to milk cartons. Once shoots emerged, seedlings in all cartons were thinned by random selection so that each carton contained one seedling. Seedlings were randomly assigned a position on greenhouse tables. Containers were wrapped in aluminum foil, and were rotated in place and into different positions on the greenhouse tables on a weekly basis to minimize differential humidity, solar radiation and heating.

Thirty representatives from each of the six populations were randomly assigned to each of three different watering treatments. Treatments approximated soil water potentials expected in high (TH), medium (TM) and low (TL) precipitation environments from the range of blue oak. Seedlings were grown from February 1997 until bud set in October 1997.

Matric potential, the adhesive forces holding water to the surfaces of soil, is the predominant component of water potential in the soil. Matric potential was measured by inserting gypsum electrical resistance blocks into the soil and leaving them undisturbed for the length of the experiment. A subsample of pots, 10 per population, were fitted with gypsum block electrical resistance soil moisture sensors to monitor matric potential in the soil (an approximation of water availability) at 20 cm of depth.

In a study done by Evans and others (1975) at the Sierra Foothills Range Field Station owned by University of California, soil moisture was monitored over a 3-year period at 4, 8, 15, 30 and 45 cm below the soil surface, using both thermocouple psychrometers and electrical resistance blocks. Soil water potential at 15 cm generally fluctuated between –0.1 and –0.8 MPa, but occasionally reached –2.5 to –5.0 MPa at 30 cm of depth. These values were used to set treatment parameters for this study. Target levels of water stress were chosen to simulate natural environments, including the arid conditions experienced by some populations, without killing off the study seedlings. Target matric potential for the three treatments was reached when water potential in pots containing the gypsum blocks reached an average of –0.1 Mega Pascals (MPa) for TH, –0.7 MPa for TM, and –1.5 MPa for TL.

Soil moisture levels in containers with the electrical resistance blocks were monitored every other day from planting until bud set in the fall. When soil moisture reached target levels of matric potential in the containers with electrical resistance blocks, water was added to all the containers in a treatment until the soil reached field capacity.

At the end of the growing season, leaves were removed, soil was carefully washed from the root mass by placing the seedlings on a screen and rinsing them with a hose using a low-velocity water stream. Plants were separated into root and shoot portions at the air/soil interface. All biomass was oven dried for three days at 45ºC.

Data for growth and biomass production of greenhouse seedlings were analyzed by among-population differences, among-treatment differences, and population x treatment interactions. The dependent variables were: root biomass (g), shoot biomass (g), shoot height (cm), root:shoot biomass ratio, total leaf weight produced by the plant (g), total plant biomass production (shoot + root + leaves) (g), diameter of stem 1 cm from air/soil interface (mm), total number of leaves produced by the seedlings, and mean leaf weight (total leaf weight/total number of leaves/plant) (g).

The first assessment applied to the data collected in seedling biomass production was to correlate precipitation normals over 30 years with means of traits examined in this study. Next, analysis of variance was performed to determine whether differences among populations were statistically significant. Finally, Bonferroni post hoc tests would determine which population differences were statistically significant. This combination of assessments would describe the pattern of genetic variation in seedling growth and biomass production among populations of blue oak.

Results

Data were analyzed with a multiple regression model (SPSS 10.0© 1999).[4] Correlations significant at $p < 0.001$ were established among the three precipitation levels for the following variables: root weight, shoot height, shoot weight, total plant weight, stem diameter, and mean leaf weight *(table 2)*.

Table 2—*Greenhouse seedlings growth and biomass production and rainfall multiple regression correlations. Standardized coefficients and partial correlations suggested that rainfall was more highly correlated than elevation for all these variables.*

Trait	**Significance (Pearson)**		**Partial correlations**		**Standardized coefficients (Beta)**	
	rainfall	elevation	rainfall	elevation	rainfall	elevation
Root weight	0.000	0.013	0.132	-0.094	0.346	-0.208
Shoot weight	0.000	0.000	0.151	-0.144	0.395	-0.318
Root:shoot	0.055	0.000	-0.045	0.091	-0.119	0.206
Leaf weight	0.019	0.068	0.041	-0.040	0.112	-0.092
Tot. plant wt.	0.000	0.006	0.133	-0.101	0.348	-0.223
Shoot height	0.000	0.000	0.202	-0.191	0.531	-0.423
Stem diameter	0.000	0.001	0.078	-0.081	0.206	-0.180
Mean leaf weight	0.000	0.000	0.034	-0.043	0.086	-0.091

[4] Mention of trade names or products is for information only and does not imply endorsement by the U.S. Department of Agriculture.

A multivariate general linear model (SPSS 10.0© 1999) found significant differences among populations and treatments for all variables (Pillai's Trace, Wilk's Lambda, Hotelling's Trace and Roy's Largest Root, all $p < 0.001$).

There was a significant effect of source populations for root weight, shoot weight, total plant weight, shoot height, stem diameter and mean leaf weight *(table 3)*.

Table 3—*Tests of between-subjects effects for blue oak seedlings growth and biomass production study.*

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
POP	wtroot (g)	74.577	5	14.915	9.029	0.000
	wtshoot (g)	1.168	5	0.234	7.620	0.000
	r:s ratio	96.578	5	19.316	2.031	0.074
	leaf wt. (g)	2.151	5	0.430	6.931	0.000
	lf+rt+sht (g)	123.609	5	24.722	9.323	0.000
	shootht (cm)	432.041	5	86.408	6.272	0.000
	diam (mm)	6.201	5	1.240	4.634	0.000
	TOT LEAF	40.406	5	8.081	0.619	0.685
	mean lfwt	0.015	5	0.003	12.766	0.000
TMENT	wtroot (g)	24.163	2	12.082	7.313	0.001
	wtshoot (g)	0.426	2	0.213	6.952	0.001
	r:s ratio	73.370	2	36.685	3.858	0.022
	leaf wt. (g)	1.689	2	0.844	13.604	0.000
	lf+rt+sht (g)	46.601	2	23.300	8.787	0.000
	shootht (cm)	18.455	2	9.228	0.670	0.513
	diam (mm)	5.692	2	2.846	10.634	0.000
	TOT LEAF	353.671	2	176.836	13.550	0.000
	mean lfwt	0.001	2	0.000	1.125	0.326
POP * TMENT	wtroot (g)	9.272	10	0.927	0.561	0.845
	wtshoot (g)	0.185	10	1.851E-02	0.604	0.811
	r:s ratio	69.848	10	6.985	0.735	0.692
	leaf wt. (g)	0.677	10	6.767E-02	1.090	0.369
	lf+rt+sht (g)	15.717	10	1.572	0.593	0.820
	shootht (cm)	104.881	10	10.488	0.761	0.666
	diam (mm)	2.245	10	0.224	0.839	0.592
	TOT LEAF	101.852	10	10.185	0.780	0.648
	mean lfwt	0.001	10	0.000	0.569	0.839

In Bonferroni post hoc tests of among-population differences *(table 4),* root weight (g), shoot wt (g), shoot height (cm), total leaf weight produced by the plant (g), total plant biomass (leaf weight + root weight + shoot weight (g), stem diameter (mm), and mean leaf weight (total leaf weight/total number of leaves produced) all were significant at $p < 0.001$ *(table 3)*.

Table 4—*Among-population differences for blue oak seedlings. Growth and biomass production characteristics—all treatments combined.*[1]

Popula-tion	N	Root weight (g)	Shoot weight (g)	Root: Shoot ratio	Total leaf biomass (g)	Total plant weight (g) (leaves+root+ shoot)	Shoot height (cm)	Stem diam. (mm)	Mean leaf weight (g)
1	87	4.38 (16,23,25)	0.459 (16,23,25)	10.51 (nsd)[2]	0.523 (16,23,25)	5.36 (16,23,25)	13.44 (16,25)	3.10 (25)	0.062 (16,23,25)
5	87	3.97 (25)	0.392 (25)	10.61 (nsd)	0.445 (25)	4.81 (25)	11.90 (nsd)	2.98 (25)	0.058 (16,23,25)
6	87	4.38 (16,23,25)	0.420 (25)	11.18 (nsd)	0.420 (25)	5.23 (16,25)	13.19 (25)	2.99 (25)	0.053 (1,25)
16	87	3.51 (1,6)	0.334 (1)	11.31 (nsd)	0.411 (1)	4.25 (1,6)	10.83 (1)	2.88 (nsd)	0.047 (1,5)
23	87	3.78 (1,6)	0.382 (1)	10.74 (nsd)	0.407 (1)	4.57 (25)	12.44 (25)	2.90 (nsd)	0.048 (1,5)
25	87	3.21 (1,5,6)	0.278 (1,5,16)	12.56 (nsd)	0.352 (1,5,6)	3.84 (1,5,6)	9.64 (1,6,23)	2.64 (1,5,6)	0.043 (1,5,6)

[1] Mean of population in cell followed by populations that are significantly different (Bonferroni, $p<0.05$)
[2] No significant differences between populations

For most traits where statistically significant differences among populations were observed, populations 1 and 6, which received at least 60 inches of rainfall normalized over 30 years, had the highest means in growth and biomass production characteristics, and population 25, with 30 year normals of 18.73 inches of rainfall had the least *(table 4)*. Significant traits included root weight, shoot weight (shown for example in *figure 2*), total plant weight (leaf + root + shoot), shoot height, and stem diameter.

The traits seedling height, stem diameter, root weight, shoot weight, mean leaf weight, total plant weight ($p < 0.001$) and total leaf biomass ($p = 0.019$) that were significantly correlated with 30 year precipitation normals indicated a strong relationship between seedling size and moisture availability. Root:Shoot ratio was not significantly correlated with 30 year precipitation normals *(table 2)*.

Tests of between-subject effects indicated significant between-treatment differences for all variables except shoot height and mean leaf weight *(table 3)*. Treatment differences in root weight, shoot weight, root:shoot ratio, total leaf weight, total plant weight, stem diameter, total number of leaves produced, and mean leaf weight were all statistically significant *(table 3)*. In general, the more water the plants received, the higher the growth and biomass production values. Conversely, root:shoot ratio generally became larger when plants received moderate or low watering treatments. Tests of between-subject effects for the population x treatment interaction term yielded no significant differences for any of the variables *(table 3)*.

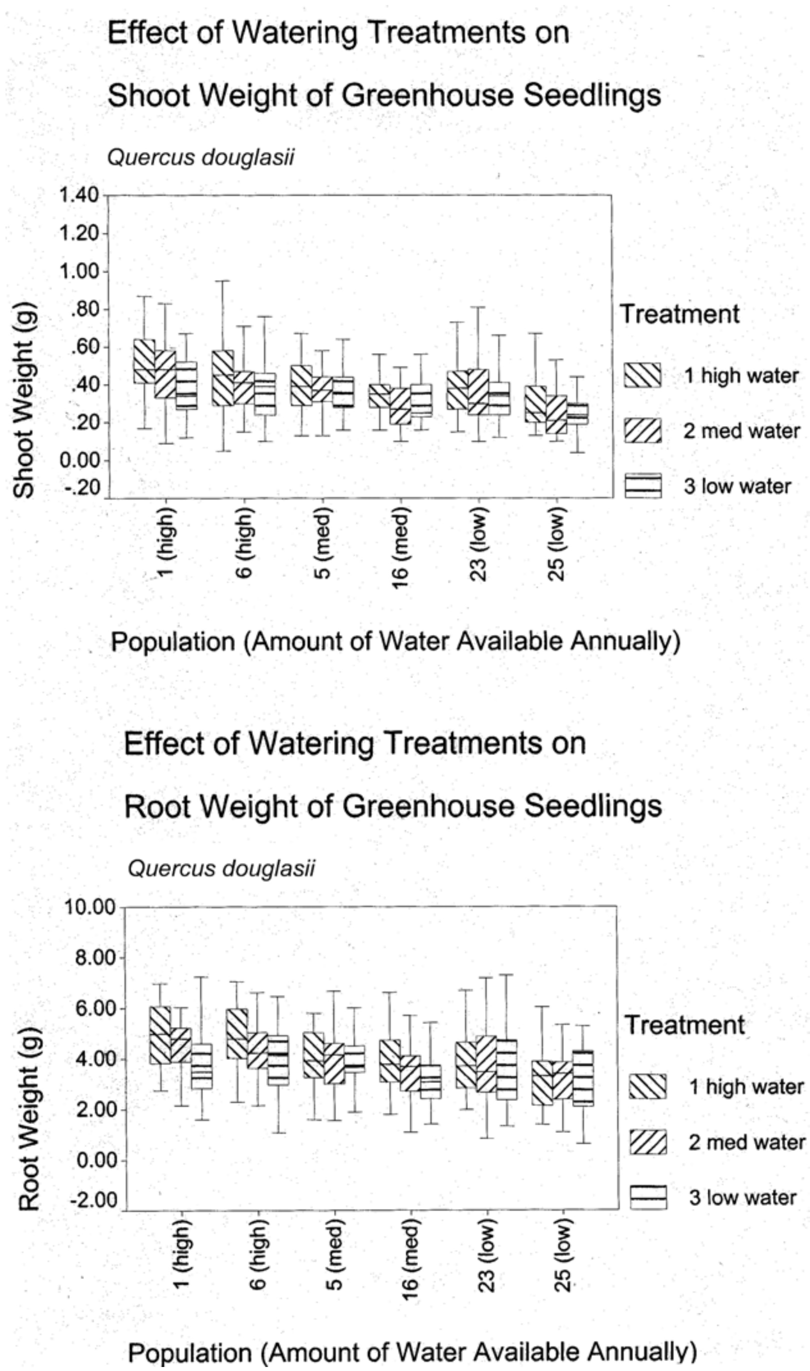

Figure 2—Plots of root weight and shoot weight for six populations of California blue oak (± range of sample).

Discussion

There were statistically significant differences among populations in growth and biomass production traits related to seedling size. Means of these traits were significantly related to water availability. Populations 1 and 6 were significantly different from populations 16, 23 and 25 for most of the traits that are significantly correlated with moisture availability. Analysis of the data suggested that seedlings from populations 1 and 6 have a higher relative growth rate than the other populations included in this study. Populations 1 and 6 are marginal populations which grow at the northern end of the range and receive as much as, or more water than other blue oak populations in California. Blue oaks in populations 1 and 6 often grow in woodlands and forests (closed canopy) where there are other tree species with which they must compete. In populations 23 and 25, in the southern end of the range, extra size does not confer any competitive advantage. There, blue oaks grow in savannas where trees are widely spaced. In those dry areas, few other tree species grow in blue oak habitat. In fact, when very low soil moisture is available, having to maintain excess biomass may be a competitive disadvantage because of the low water resources.

This study provided evidence to suggest that in general, blue oak seedlings have the ability to produce more biomass when more water is available but interaction plots did not show trends of populations responding to treatments differentially (statistically significant interactions), which would have provided evidence that particular populations respond differently to water availability (e.g. local adaptation in plasticity). The interaction plots for all variables showed consistent trends for all populations when compared across treatments. These findings indicate that plasticity in traits examined has not been selected for at the population level; i.e. the magnitude and direction of plasticity does not differ for some populations relative to others, for the soil moisture levels chosen for this study. There is difficulty in selecting levels of water stress that are high enough to force experimental seedlings to reveal adaptive traits without causing so much mortality that the research cannot be completed. Perhaps a study that has a range of levels of extreme water stress (perhaps the low end could begin at –1.5 MPa and the high end could be set at –3.0 MPa) may reveal adaptive traits or plasticity not detected in this study. Treatments of higher water stress could mimic natural selection by eliminating individuals that are not adapted to more extreme conditions of water stress.

Although this study specifically examined among-population differences related to the precipitation gradient in California blue oak habitat, it is likely that traits reflect a combination of environmental gradients as well as the water availability gradient. Maternal effects in blue oak have also been shown to be an important source of variation in acorn and seedling characteristics (Rice and others 1993). Analysis using a general linear model suggested that all populations for at least some of the traits had high levels of within-population variation, implying that there is variation either within or among individuals in a population, or both. This helps to explain the extensive range of blue oak and the varieties of climatic and community characteristics the species can endure.

Not all traits examined in the greenhouse study were correlated with water availability. This finding reflects the complexity of growth and biomass production in oaks, which is surely the result of combinations of many genetic and environmental factors, and their interactions. Elevation has a pronounced effect on length of the growing season. Plants at higher elevations are more subject to frost damage or mortality, and so generally have a restricted growing season relative to lower elevation plants. In addition, plants at higher elevations generally receive more precipitation than their counterparts at the same latitude but at lower elevations. Latitude, at least in an indirect manner, affects many factors including day length, temperature regimes, seasonal characteristics, and growth period as well as water availability.

In summary, there are significant differences among populations related to water availability in some seedling size and biomass production traits. However, other among population differences are likely to be diluted or indistinct in traits that result from interrelated environmental and genetic factors.

Conclusion

Seedlings of blue oak examined in this study exhibited ample variability in growth and biomass production traits within and among populations. This variability, in combination with the species' drought-avoidance and drought-tolerance processes, allows blue oak to be continuously distributed over such a broad expanse of area and ecological conditions in California. Blue oak seedlings demonstrated high within-population variability for many of the traits examined in this study, and significant among-population variability as well. Since seeds from the more mesic areas tended to yield seedlings that produced more biomass, perhaps seed source zone maps should be used for blue oak restoration or planting projects until evidence is produced that coadapted or regional gene complexes do not exist in this species.

Significant among-population differences existed in the variables root weight, shoot weight, leaf weight, total plant weight, shoot height, stem diameter and mean leaf weight. All these traits were significantly correlated with the water availability gradient. Because size of seedlings from populations 1 and 6 were significantly different from populations 16, 23 and 25 for most traits, these findings suggest that size may contribute to seedlings' ability to respond to high water availability.

The three treatments caused the means of each of these variables to differ significantly: Shoot weight, root weight, root:shoot ratio, leaf weight, total shoot weight, stem diameter, total number of leaves produced, although treatments did not cause significant differences in shoot height or mean leaf weight, probably because there was so much variation in each population.

The differences caused by treatments suggest that blue oaks have the ability to respond plastically, to some extent, to different levels of water availability. This finding is in agreement with Rice's finding of phenotypic plasticity in WUE of blue oak seedlings (1996).

Although traits that exhibited among-population differences were significantly correlated with the water availability gradient, it is likely that other environmental characteristics not examined in this study, such as temperature and day length, act in conjunction with water availability as selective forces. This study also did not assess the effects of combinations of environmental characteristics, for instance, temperature and precipitation, acting together. Further study in these areas would help continue to elucidate the geographic variation in populations of blue oak.

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