AN ABSTRACT OF THE THESIS OF

Andrew David Bower for the degree of Master of Science in Forest Science presented on January 15, 1998. Title: Response of Annual Growth Ring Components to Soil Moisture Deficit in Young, Plantation Grown Douglas-fir in Coastal British Columbia.

Abstract approved: ______________________________
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The goal of this study was to determine whether annual growth ring variables of young coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco.) growing in progeny tests are sensitive to availability of soil moisture during the growing season. If ring variables are found to respond to soil moisture deficit (SMD) in consistent and predictable ways, they might prove useful for screening genotypes for drought hardiness in breeding programs. Increment cores were collected from 16 (18-19 year-old) trees on each of eight progeny test sites in coastal British Columbia. X-ray densitometry was used to measure eight variables (earlywood and latewood width and density, latewood proportion, maximum density, latewood mass, and total ring mass) on eleven annual growth rings (years 1985-95) of each core. Regression analyses revealed that all ring variables are strongly influenced by ring distance from the pith (age trends). In addition, after accounting for age-trends, all ring variables were significantly associated with SMD, although the associations were often complex (i.e., involving second and third order polynomials of SMD) and differed significantly across sites. Linear trends in four ring variables (latewood density, proportion, and width; and total ring mass), with increasing SMD, were as expected and were consistent across sites; and thus, show promise in screening for drought hardiness. The remaining ring

variables showed inconsistent associations with SMD across sites. A small companion study on cell morphology suggested that latewood cells increase in density with increasing SMD due to decreased cell lumen diameter, and not because of increased cell wall thickness.

Response of Annual Growth Ring Components to Soil Moisture Deficit in Young, Plantation Grown Douglas-fir in Coastal British Columbia

by

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APPROVED:

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Head of Forest Science Department

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Andrew D. Bower

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CHAPTER 1 - THESIS INTRODUCTION

Introduction

This project was funded by the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) and is a part of their ongoing research program studying the genetics of adaptation of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco.) to stress environments. Adaptation of a tree species to its environment is very important and is of great concern to tree breeders. Even varieties that have undergone genetic improvement for growth and other economically important traits will suffer if they are planted on sites to which they are not well adapted, resulting in less than optimal growth, and possibly, death. As the goal of most tree breeding programs is to increase tree growth and yield of timber, the importance of adaptation is clear.

Native forest tree species in the Pacific Northwest, including Douglas-fir are generally well adapted to the climate on a regionwide scale. The natural range of Douglas-fir in this region, however, spans a wide range of latitude and elevation, and as a result, local microclimatic conditions are widely variable, and can be quite harsh. Trees, for example, may grow naturally, or might be planted, in frost pockets that expose the trees to temperatures well below what is experienced normally. In dry areas such as southwestern Oregon, and in areas that experience a rain shadow, such as the eastern (leeward) side of the Olympic mountains, Vancouver Island and coastal eastern (leeward) side of the Olympic mountains, Vancouver Island and coastal mainland British Columbia, trees can be subject to severe drought stress. These potential extremes in climatic conditions make the study of adaptation of Douglas-fir to environmental stresses a number one priority of the PNWTIRC.

This project is the first stage of a two-stage study designed to investigate the presence and degree of genetic variation in drought hardiness of Douglas-fir growing in field progeny tests. The first stage was intended to determine whether impacts of summer drought could be assessed from annual growth ring variables in young trees. Measurements of various components of annual growth rings were compared with climate data to determine if one or more of these components are sensitive to drought. The second stage of the study will investigate growth ring components in a large sample of full-sib families growing on a single study site to evaluate family differences in drought sensitivity.

Thesis Organization

This thesis includes four chapters, followed by appendices: Chapter one is this introduction, chapter two presents the main results of the study, chapter three describes a small side investigation on the impacts of drought on morphology of Douglas-fir xylem cells, and chapter four summarizes the main conclusions of the thesis and provides recommendations for future research. Chapter two follows the format of a scientific journal article. The appendices include supplementary data and information supporting the methods and results discussed in the thesis.

CHAPTER 2 - RESPONSE OF ANNUAL GROWTH RING COMPONENTS TO SOIL MOISTURE DEFICIT IN YOUNG, PLANTATION GROWN DOUGLAS-FIR IN COASTAL BRITISH COLUMBIA.

Introduction

The coniferous forests of the Pacific Northwest of North America have evolved in response to the moisture and temperature conditions, as well as nutrient regimes common to a maritime, winter-wet, summer-dry climate. Although Douglas-fir (*Pseudotsuga Menziesii* [Mirb.] Franco.) is well adapted to this climate, in some locations the degree and duration of stomatal opening is limited due to seasonal reductions of available soil water and increased evaporative demand. While this response to drought helps individuals to conserve water by limiting evaporative losses, it also reduces the amount of carbon dioxide uptake, subsequently reducing photosynthetic rate, tree growth, and wood production (Waring and Franklin, 1979).

A goal of most commercial forestry operations is to maximize total growth and production of wood in a stand. Thus, most tree improvement programs place a large emphasis on improved stem volume growth, and any factor that threatens to reduce potential tree growth is of great interest to tree breeders. Although it is known that climate plays a significant role in tree growth, responses of young Douglas-fir to conditions where soil moisture is limited are not well understood, including the degree to which these responses are under genetic control. Understanding genetic mechanisms involved in drought hardiness is essential to developing methods of screening for drought hardiness and for developing varieties specifically adapted to dry sites.

Screening for drought hardiness at the seedling stage may be of particular importance because trees are most susceptible to damage or death from high soil moisture deficit levels (calculated as the difference between potential and actual growing-season evapotranspiration) at this stage. Nevertheless, impacts of drought in older trees, especially after the onset of inter-tree competition is also of concern. In addition, the large number of Douglas-fir progeny tests established in the region (Woods, 1993), means that field grown families are readily available for assessment. Thus, reliable methods of screening genotypes for drought hardiness in older trees (i.e. beyond the seedling stage), would be of great benefit to Douglas-fir breeding efforts.

It is well known that during the growing season, tree growth is greatly influenced by the available water in the soil, and in some forested areas this can be the most important growth limiting factor (Armson, 1977, pp. 257). Water available to trees is the amount of water held in the soil at water potentials between field capacity and permanent wilting point. Variation in water availability is determined by the amount of precipitation, solar radiation, soil texture, competing vegetation, drainage and other factors (Armson, 1977, pp. 60).

Because of the significant role of climate on the annual growth of trees, characteristics of annual rings are correlated with variation in climate (Jordan and Lockaby, 1989). The science of dendrochronology makes use of the annual variation of growth rings to reconstruct climatic patterns (Chang and Aguilar, 1980). Relationships between annual growth rings and soil moisture availability have been reported for several, species such as Douglas-fir (Robertson and Jozsa, 1987 and 1988; Robertson et al, 1990), loblolly pine (*Pinus taeda* L.) (Bassett, 1964; and Woods and Debrunner, 1970), shortleaf pine (*P. echinata* Mill.) (Bassett, 1964), red pine (*P. resinosa* Ait.) (Zahner and Donnelly, 1967), and ponderosa pine (*P. ponderosa* Laws.) (McLeod and Running, 1988).

Fritts (1976, pp. 19) states that "in studies of ring widths and drought it is important to rely upon trees growing in the driest sites, for those are the individuals in which ring width is most likely to have been limited by drought." Previous studies linking annual ring growth variables with climatic data in Douglas-fir have accordingly been most successful when applied to very old trees, growing in extreme environments. For example, Robertson and Jozsa (1988), using dendrochronology to reconstruct climate, found statistically significant relationships between climate and growth in 300-year-old Douglas-fir growing in a harsh environment in Banff, Canada. In another study, a similar approach was applied to younger (50 year-old), more vigorous Douglas-fir trees, in more moderate environments, but statistically significant relationships between growth rings and climate factors were not found (Robertson and Jozsa, 1987). Later, a more realistic environmental model was used, that took into account not only climatic factors, but also several site specific parameters that can affect tree growth. With this expanded model, Robertson et. al. (1990) were successful in relating growth ring variables to climate using data from 70 year-old Douglas-fir trees growing in even-aged stands on Vancouver Island. These authors measured eleven components of annual growth rings with X-ray densitometry, and examined the relationships of these growth ring components with 16 separate climate variables. Of particular interest was the finding that annual soil moisture deficit (SMD), calculated from a water balance model, accounted for 51% of the annual variation in ring weight. Spittlehouse (1985) also found a close relationship of ring growth (annual basal area increment) with both SMD and annual basal area increment for the 15 years of his study in young (15-30 year-old) Douglas-fir growing on Vancouver Island.

Bassett (1964) studied differences between measured growth of loblolly and shortleaf pines in a second-growth stand in southeast Arkansas, and calculated potential growth based on soil moisture availability. Soil moisture content was estimated for each day during the growing season from 1940 through 1960 and combined with estimates of potential evapotranspiration, to calculate an index of potential growth: the calculated growth-day unit = 1 - (P x T), where P is potential evapotranspiration, and T is mean moisture tension in the top foot of soil. Days in which this index was positive were classified as "growth days" and days in which it was near zero, or negative were classified as "no growth days". He found that diameter growth of individual trees is closely associated with soil moisture availability, and that linear regressions of measured growth (in both basal area, and cubic foot volume) on the number of growth days and of no growth days explained 95-97% of the variation about the regression line.

In a study investigating the relationships of annual radial growth of loblolly pine in east Texas (dependent variable), with 48 climate and soil (independent) variables, only three independent variables were retained in a stepwise multiple regression analysis (Chang and Aguilar, 1980). The annual number of days in the current year with more than 0.25 mm of rain and total summer precipitation (May-October) of the previous year were both positively correlated with annual radial growth, while the difference in maximum air temperature between July and January was negatively correlated. These three variables explained approximately 40% of the total variation of radial growth. Inclusion of soil type as dummy variable along with the three climatic variables in a covariance analysis, made it possible to explain an additional 10% of the variation in annual radial growth, increasing R^2 from 0.40 to 0.50. In another study in loblolly pine, Cregg et. al. (1988) found that during the early part of summer, growth was positively related to mean daily temperature, while later in the summer, growth was negatively related to mean daily temperature. In addition, latewood percentage was nearly 10% lower in the drier of the two years of the study. Friend and Hafley (1989) observed that annual growth of loblolly pine increased with spring temperatures and summer soil water availability, yet is also sensitive to climate in the previous fall; i.e., a climate that favors production and accumulation of photosynthates in the fall results in increased cambial growth the following spring. They reported that cambial growth was positively associated with increased soil water in the previous fall, but was negatively associated with the number of rainy days in that season. The latter result was unexpected; they postulated that successive cloudy days in late summer and fall reduces photosynthesis, resulting in lower carbon stores for the following year.

Zahner and Donnelly (1967) studied variation in ring width of red pine (*Pinus resinosa* Ait.) and rainfall patterns in lower Michigan. In two 21 year-old plantations, they found a high correlation between mean ring width and total annual rainfall (previous year, current year, and sum of the two) and total annual SMD (previous year, current year, and sum of the two) over a 10-year period. They reported that moisture deficit for the current year had a simple correlation of -0.83 with ring width, while rainfall for the current year had a correlation of 0.69. Multiple linear regression including both rainfall and moisture deficit of both the previous and current growing seasons accounted for over 80% of the variation in ring width.

The objective of the study described in this chapter was to determine the extent to which annual growth ring components of young, plantation-grown Douglas-fir are sensitive to drought conditions during the growing season. This was investigated by comparing measurements of eight different annual growth ring components with estimated annual SMD's over 11 years, for 18 and 19 year-old trees growing in six progeny test sites on eastern Vancouver Island and coastal mainland British Columbia. Annual growth ring components were measured by X-ray densitometry of increment cores, and a repeated measures type of analysis used to regress each growth ring variable on annual SMD, while accounting for age-trends. Four ring variables, latewood density, latewood proportion, latewood width, and total ring mass, appear to be sensitive to SMD, and were consistently related to SMD across the six test sites. These growth ring variables may be useful as indicators of sensitivity to SMD, and hence drought hardiness of individual genotypes or families.

Materials and Methods

Site Description and tree sampling

In order to sample trees subjected to a range of annual SMD, the eight progeny test sites were chosen to include a variety of elevations, slopes (percent, aspects, and position) and soil types of dryer locations in rain shadow areas within the Coastal Western Hemlock biogeoclimatic zone (Ministry of Forests, 1988) of the east coast of Vancouver Island and coastal mainland (southwestern) British Columbia. (Figure 2.1, Table 2.1).

Fig 2.1. Locations of the eight progeny test sites included in the study.

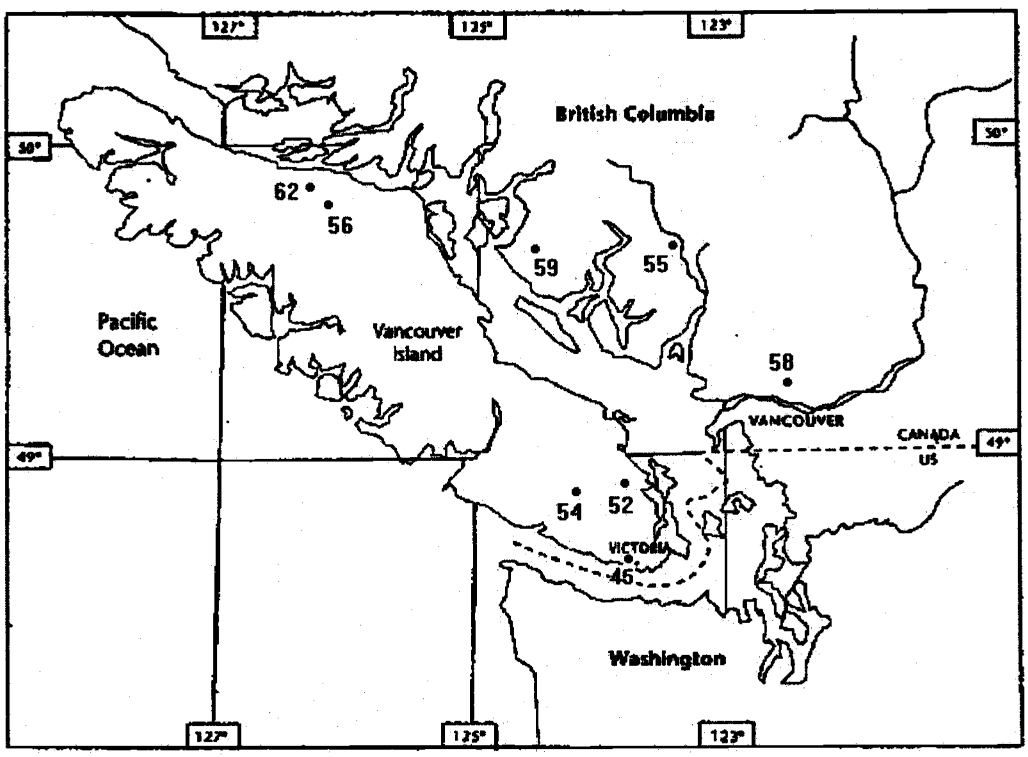

Table 2.1. Topographic and soil data for the eight progeny test sites included in the study.

Site	Name	Longitude	Latitude	Elev. (m)	Slope			Soil		
					%	Aspect	Position	Type	Root zone depth (cm)	% stone
45	Kirby Creek	48° 25' 40"	123° 55' 50"	364	15	N	Upper	Loam	70	15
52	Mt. Prevost	48° 49' 15"	123° 49' 00"	273	5	S	Flat	Clay- loam	50	35
54	Hatton Creek	48° 47' 40"	124° 32' 55"	209	25	N	Mid	Sandy- loam	50	15
55	Squamish River	50° 12' 05"	123° 22' 30"	560	20	ESE	Lower-mid	Sandy- loam	70	15
56	Compton Creek	50° 13' 15"	126° 09' 45"	530	20	SSE	Lower-mid	Sandy- loam	55	35
58	Steelhead	49° 12' 00"	122° 17' 30"	394	25	NNE	Mid	Sandy- loam	100	10
59	Freeda Creek	49° 52' 35"	124° 15' 25"	209	5	S	Toe	Loamy- sand	70	< 5
62	Eve River	50° 19' 20"	126° 14' 10"	258	15	SE	Lower	Loam	60	40

The progeny tests were established by the British Columbia Ministry of Forests with full-sib families from two series of crosses; one (series 5) completed in 1977, the other (series 6) in 1978. All sites were planted at a 3m x 3m spacing, with two year-old seedlings. Series 5 sites (45, 52, 54, and 55) were established in 1979, and series 6 sites (56, 58, 59, and 62) in 1980. Therefore, at the time of sampling (June, 1996) the trees were 19 (series 5) and 18 (series 6) years old from seed. Increment cores were extracted from 16 trees on each site. In order to ensure a broad sample of genotypes, cores were taken from two trees of each of eight families, with the same eight families sampled on all four sites of a series. Families were selected at random, with all individuals sampled within the same block, or adjacent blocks, to minimize microsite variation. The two largest, healthiest trees representing a family within this area were sampled, in order to obtain samples that contained the maximum number of rings, and were representative of the potential growth on a site.

Five millimeter increment cores were taken in June of 1996, at approximately one meter above the ground, to avoid basal swelling, but to maximize the number of annual growth rings sampled. On seven of the eight sites, cores were run straight through the tree, producing two pith-to-bark cores. On site 54, the first site sampled, only one pith-to-bark increment core was extracted. Diameter at breast height (DBH) was also measured on each tree at the time of coring, and site means for DBH and tree height at age 11 were supplied by the B.C. Ministry of Forests (Figure 2.2). These measurement are indicative of differences in site productivity, which potentially could lead to differences in response to drought. This is discussed further in the results.

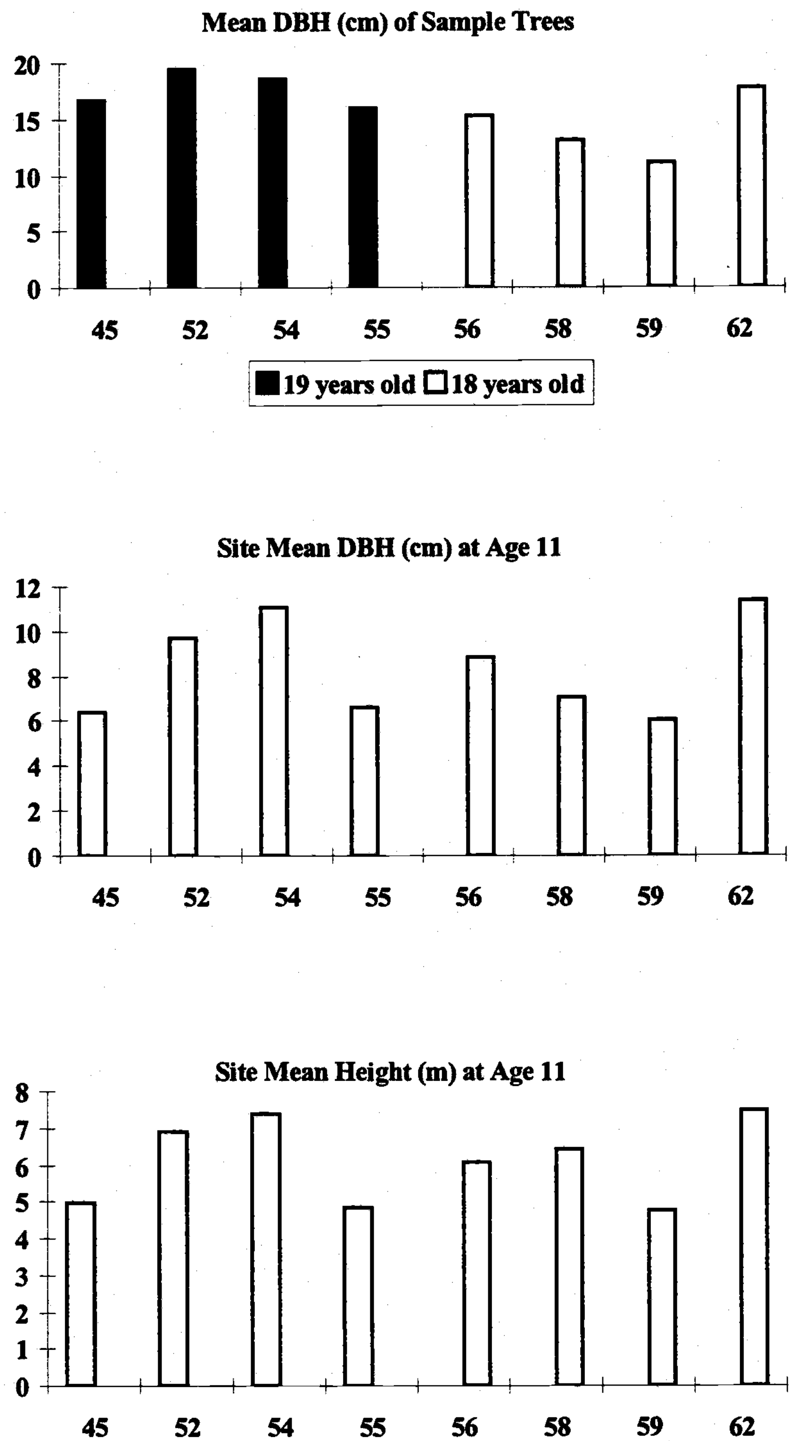

Figure 2.2. a) Mean stem diameter (DBH) of the 16 trees sampled on each site in 1996 (ages 18-19), b) Mean DBH and c) Mean height of all trees on each site at age 11.

Core Preparation and X-ray densitometry

The cores were oven dried, cut into a flat strips approximately 1.5 mm thick using a saw with parallel mounted circular blades, and soaked in a 95% ethanol/toluene solution to remove any extractives (Appendix 1). The cores were then air dried and allowed to equilibrate to the ambient moisture content of the room in the Oregon State University, Forest Research Laboratory, where X-ray densitometry was performed. This X-ray densitometer uses a direct reading Enraf/Nonius collimated tube type X-ray generator and photon energy discriminating radiation detection system. A count of the number of X-rays that pass through the sample is recorded at 100 μm increments along the length of the core. The ratio of the number of X-rays passing through the sample to the number that pass through air only is used to calculate the density of the sample at each point according to Beer's Law (Appendix A). As the trees were 18 and 19 years old at the time of sampling, most cores contained approximately 15 annual growth rings. Measurements on the first two to three rings were discarded, however, because the first ring was usually too small to measure accurately, and the measurements on the next 1-2 rings are incorrect if the core does not hit the pith perfectly. In addition, the 1996 growth ring was not complete at the time of sampling. This left 11 growth rings, 1985 through 1995, included in all the analyses.

Annual Growth Ring Measurement

The density values calculated from the densitometer output were analyzed using the Dendroscan tree-ring width and density measurement system software (Varem-Sanders and Campbell, 1996). Dendroscan uses the fluctuations in the density profile along the core to delineate growth rings (Appendix Figure A.1). The boundary between successive growth rings is placed at the steepest part (inflection point) of the density curve. Dendroscan occasionally interpreted anomalies in the wood, that lead to sharp changes in density between adjacent sample points, as inflection points, and incorrectly placed a growth ring boundary at this location. As is standard procedure, plots of the density profiles determined by the software were manually reviewed and edited where needed to ensure that ring boundaries were placed correctly. The earlywood/latewood boundary within a single growth ring is placed at the location of the average of the minimum and maximum densities within a single year. The software was then used to generate values for annual growth ring components. Values were generated for total ring width (TRW), earlywood width (EWW), latewood width (LWW), earlywood density (EWD), latewood density (LWD), maximum latewood density (MXD), and latewood proportion (LWP) for the years 1985-95 for each core. In addition, from these data, total ring mass (TRM) (ring volume for a 1 cm thick section x average ring density) and latewood mass (LWM) (latewood volume for a 1 cm thick section x latewood density) were calculated. All of the above growth ring variables, with the exception of TRW (eight total), were subsequently related to SMD using regression. Plots of several of the variables, however, showed increasing variation with increasing age. Thus, to stabilize error variance over age, a natural log transformation was applied to TRM, LWM, LWW, EWD, and EWW prior to analysis.

Climate Data

As a composite measure of the factors that influence moisture availability in soil during the growing season, total annual SMD is a useful parameter. SMD measures the magnitude of stress trees may be under due to lack of water. SMD was calculated from a water balance model (Giles et al., 1985), and is represented by $E_{max} - E_t$ (Figure 2.3), where E_{max} is the calculated maximum evapotranspiration possible at the observed level of radiant energy, and E_t is actual evapotranspiration given weather and site conditions. If the level of soil water is at or near field capacity, actual evapotranspiration is limited only by net radiant energy, in which case $E_t = E_{max}$. If the supply of water in the soil is limited (under drought conditions), then $E_t < E_{max}$, and the larger the value of SMD, the less moisture is available.

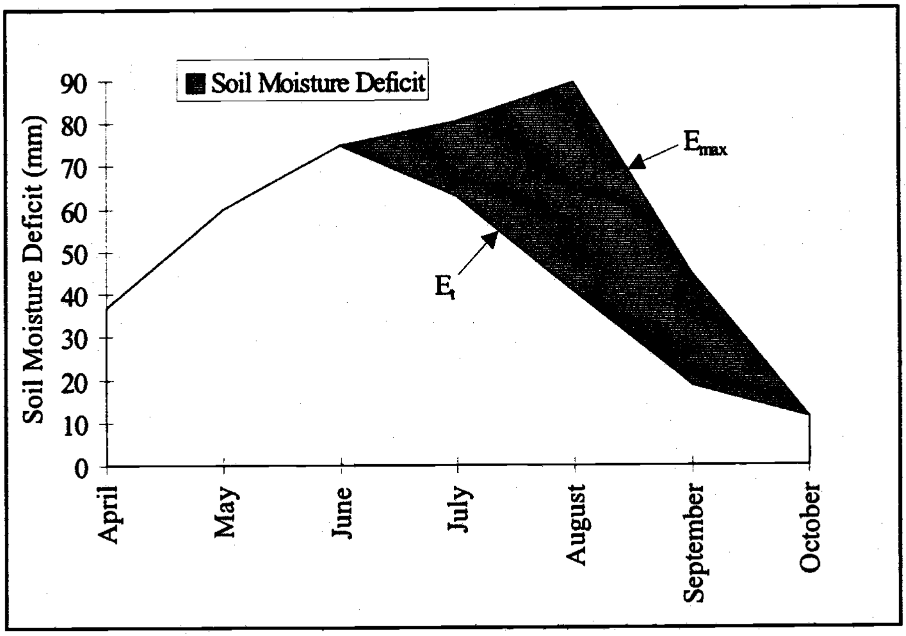

Figure 2.3. Monthly estimates of SMD for site 59 in 1985.

The water balance model uses inputs of climate and site data to estimate E_t, E_{max}, and monthly SMD (Appendix tables B.1 and B.2a - B.2h). Total annual SMD was calculated as the sum of monthly SMD's for April - October. There is no SMD during other months in the year because the soil is recharged by late fall and winter rains. The climate data required are mean minimum and maximum monthly air temperatures, total solar radiation, and total rainfall. The climate data were obtained from nearby weather stations. When no weather station was close enough to have experienced the same weather conditions as a study site, data were interpolated between other stations. Site data required in the water balance model, percent slope, aspect, latitude, root zone depth, soil type and percent stone content (Spittlehouse and Black, 1981; Spittlehouse, 1985; Giles et al., 1985; and Robertson et al., 1990), were collected for each site at the same time increment cores were sampled (Table 2.1). The water balance model was developed, in part, by Dr. David Spittlehouse of the Research Branch, British Columbia Ministry of Forests, and he calculated the SMD values used in this study based on the site locations and site data that we supplied. Two of the sites (45 and 58) were not included in further analyses because SMD was either zero or very small in all years included in the study (Figure 2.4)

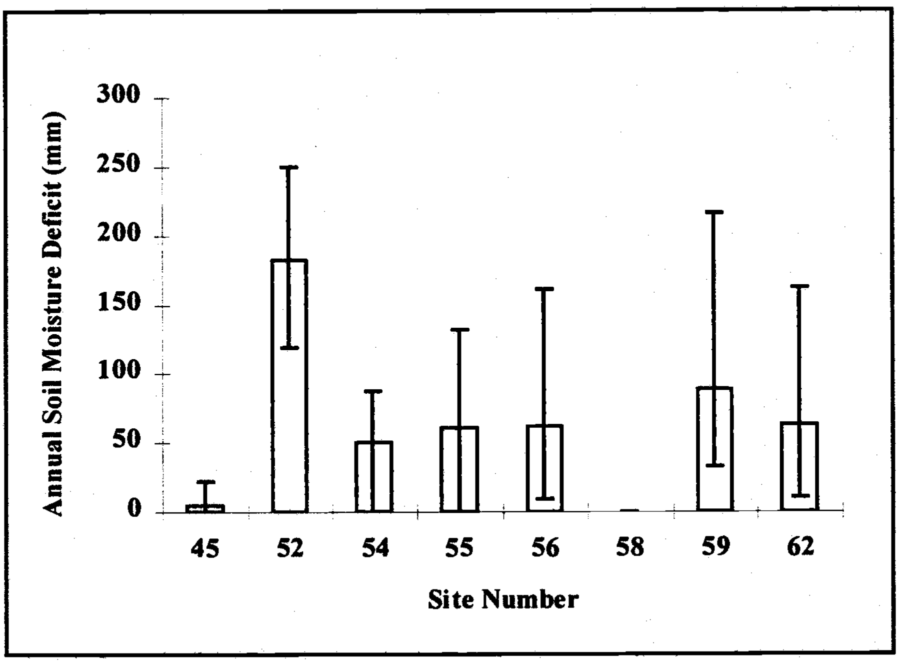

Figure 2.4. Mean total annual soil moisture deficit (SMD) values over 1985-95 (range in brackets) for eight progeny test sites on Vancouver Island and Mainland British Columbia.

Statistical Analysis

Our main interest is in the pattern, strength and consistency of the relationships between growth ring variables and SMD. These relationships were analyzed by regressing measurements of the growth ring variables on SMD. Growth ring variables, however, are influenced by factors other than soil moisture in this study (e.g., site, family, ring age), which then need to be included as independent variables in the regression. In particular, ring variables in young trees are expected to change in a consistent fashion with age (i.e., with increasing distance from the pith), as trees become established after planting and are first free-to-grow, and then are influenced by tree-to-tree competition. When modeling growth ring data in older trees, the regression of ring width on age usually follows a negative exponential function. This function, however, is inadequate for young trees, where ring width usually increases for the first 10-30 years (Fritts, 1976, pg. 263). The inadequacy of a negative exponential function was indeed the case in this study for not only ring width, but all of the annual growth ring components measured; the general form of the age-trend relationship was found to be either an upward trending straight line or a curvilinear function. To account for these age-trends in ring variables, appropriate linear or polynomial terms for age were included as parameters in the regression equations.

In addition to age-trends in growth ring variables, expression of traits in any one year are related to their expression in both the preceding and succeeding years, resulting in observations that are serially correlated (Meredith and Stehman, 1991). Therefore, all regressions employed repeated measures analysis using the MIXED procedure in SAS

(SAS Institute, Inc., 1997), REML estimation, and a first-order autoregressive covariance structure specified (Appendix F). This covariance structure is useful for data where neighboring values are strongly related, but as values get further apart the strength of the relationship decreases, as with yearly measurements on growth rings.

Preliminary regression analyses utilized data for each of the six sites individually to examine the general form of age-trends and relationships between growth ring variables and SMD. These analyses indicated that quantitative relationships of growth ring variables with age and SMD can be adequately expressed by multiple regressions employing cubic or lower-ordered polynomial terms. Differences in curve shape and slope in these analyses also indicated that the relationships with both age and SMD differed by site (i.e. significant age x site, and deficit x site interactions may exist). In order to account and test for these interactions, joint regression analyses were carried out for all growth ring variables using growth ring data pooled over all six sites. The three sites in series 5 contained a different set of families from those in series 6, so series was accounted for as a block effect with sites nested within series. Ultimately, four sets of independent variables were used in the regression analyses: a) source variables, including series and sites within series b) age-trend variables, including age, age^2, and age^3, and their interactions with site within series, c) relationship variables including family nested within sites and trees nested within families, and, d) SMD variables, including deficit, $deficit^2$, and $deficit^3$ and their interactions with site within series. Source, age-trend and SMD variables were included as fixed effects in the model, while relationship variables were considered as random effects.

Model building was done in three stages. Ring variables were first fitted to source and age terms, relationship terms were added next, and finally SMD terms were added, running the model after each addition to test the significance of the added terms. In the first (age-trend) stage, each ring component was fitted to a model that included source variables; age-trend variables, including terms for the highest-ordered polynomial (e.g., quadratic or cubic) found necessary in preliminary regressions to fit the data for any one site; and, interactions of all age-trend terms with site within series. A backwards elimination procedure was used to select the best fitting "age-trend" model. Highest ordered interactions were tested first and removed if non-significant, then the next highest ordered term was tested, and so on, until terms could no longer be removed. An F-test that is available in the SAS PROC MIXED output was used to test for significance of each individual term. As this was an observational study, an α = 0.10 level was used for significance tests. Any main effect terms that were included in a significant interaction term were retained. This process determined which age-trend and age-trend x site interaction terms were included in the final model. The source variables were retained in the model regardless of their significance, in order to account for the differences between sites and the sets of genetic materials used in the two series, as a result of the sampling structure.

In the second stage, terms for family and tree within family were added to the "age-trend" model terms and tested using a forward selection procedure. A term for family within site was added first, and its significance tested by comparing the log likelihood of the new model to that of the previous (age-trend) model, where family effects were not accounted for. The value -2[log likelihood (previous model) - log likelihood (new model)] has a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters in the two models (1 in this case) (SAS Institute Inc., 1997 pg. 651). If this χ^2 statistic was significant ($p \leq 0.10$), the term for family remained in the model. A term for trees within families was then added and tested in the same manner.

In the final model-building stage, terms for SMD were added to the previous model terms. A backwards elimination procedure similar to that employed for the age-trend terms was used to pare down the full model to its final form. Like the age-trends, the highest ordered polynomial indicated from the preliminary individual-site analyses was used as the starting point for the SMD and SMD x site terms. Again, any main effect SMD terms that were included in interaction terms were left in the model regardless of their significance.

The sampling design in this study was not intended to allow for a meaningful assessment of genetic effects. Nevertheless, terms for families and trees within families were included in the final models (if significant), in order to help account for variation in the expression of ring variables. Interactions involving relationship variables and other independent variables in the model were not considered because these interactions would have added unnecessary complexity to the regression models. For the same reasons, interactions involving age and SMD were not considered, although it is acknowledged that age x SMD interactions may be biologically meaningful. This will be further addressed in the Results and Discussion section.

In order to be able to use ring variables to assess sensitivity of trees to drought in a reliable manner, association of ring variables with SMD should be relatively consistent across sites. The final regression models derived by the above procedure, however, often included polynomial terms for SMD and their interactions with sites, making it difficult to determine whether associations of ring variables with SMD were generally increasing, decreasing, or were inconsistent over sites. To aid in interpretation, therefore, the regressions were re-run using orthogonal polynomials for SMD (Draper and Smith, 1966). The intent here was to derive independent regression terms for describing the underlying linear trends of ring variables with SMD on each site, and to test for interactions in these linear trends across sites.

Results and Discussion

General Composition of Fitted Regression Models

All final regression models included terms for each of the four classes of independent variables, although the form of the regressions on age and SMD, and the presence of age x site and SMD x site interaction terms, varied by ring variable (Table 2.2). Given large differences in site productivity (Figure 2.2, Table 2.2), it is not surprising that site (series) proved to be a highly significant source of variation in all ring variables. The productivity of the site is a function of many different influences, but a clear effect of SMD is evident by comparing the values of the site means of the annual growth ring variables with the mean SMD on each site. For all of the variables except LWP and TRM, the range in site means for series 5 are always smaller than those for series 6 (Appendix Table A.1). The ranges of SMD's experienced on the sites in series 5 are correspondingly more limited (Figure 2.4). Within series 6, site 59 has the highest mean SMD, and also the highest MXD, LWD, and EWD, as well as the smallest LWW and EWW, however these relationships were not consistent within the sites in series 5. Both relationship variables [family(series) and tree(family series)] proved to be significant for all response variables, indicating that genetic and/or microsite variation also has a strong influence on annual ring components. In fact, the range of family means within sites were greater than the range in site means for all growth ring variables analyzed, while the range of the means for each of the eleven rings in each core within a site varied to an even greater extent. For example, for LWD, the site means ranged from 0.784 to 0.912 (0.128), while the family means ranged from 0.716 to 0.965 (0.249), and the ring means within a site ranged from 0.719 to 1.071 (0.353) (Appendix Table A.1).

Regression on age, in all but one case (MXD) involved cubic terms, and in all cases involved one or more interactions of age variables with site, indicating complex relationships of ring components with age. Likewise, regressions of ring components on SMD were complex, often involving either second or third degree polynomial terms and interactions with site (Table 2.2).

Table 2.2. Independent variables included in final fitted regression models for each of eight annual growth ring components.

Independent Variables	Annual Growth Ring Component							
	LWD	LWP	ln (LWW)	ln (LWM)	ln (TRM)	MXD	ln (EWD)	ln (EWW)
SOURCE								
Series	X	X	X	X	X	X	X	X
Site(series)	X	X	X	X	X	X	X	X
AGE								
Age	X	X	X	X	X	X	X	X
Age^2	X	X	X	X	X	X	X	X
Age^3	X	X	X	X	X		X	X
Age x Site(series)	X	X	X	X	X	X	X	X
Age^2 x Site(series)	X	X	X	X	X		X	X
Age^3 x Site(series)	X	X	X	X			X	X
SMD								
SMD	X	X	X	X	X	X	X	X
SMD^2	X	X	X	X	X	X	X	X
SMD^3		X	X		X	X	X	X
SMD x Site(series)	X	X	X	X	X	X	X	X
SMD^2 x Site(series)	X	X	X		X	X	X	X
SMD^3 x Site(series)						X	X	X
RELATIONSHIP								
Family (series)	X	X	X	X	X	X	X	X
Tree (family series)	X	X	X	X	X	X	X	X

[a] LWD = latewood density
LWW = latewood width
LWM = latewood mass
TRM = total ring mass
MXD = maximum density
LWP = latewood proportion
EWD = earlywood density
EWW = earlywood width

Age trend determination

Strong age trends were found for all eight ring variables investigated, supporting the conclusion of Vargas-Hernandez et al. (1994), that distance from the pith is a major factor influencing annual growth ring components in young Douglas-fir, although the significant age x site interactions in all cases indicate that the shapes of curves differed across test sites (Table 2.2). Plotting predicted age trends of ring variables for each site, together with corresponding sites means for each age, not only reveals how age trends differed across sites, but also how well the age-trend regressions fit the data. The regression models appear to do an adequate job in accounting for age effects; illustrated for three ring variables in Figures 2.5a - 2.5c, and for the remaining variables in Appendix Figures C.1a - C1.e. Although age-trend regressions differed significantly across sites, the general form of the age-trend for each ring variable was relatively consistent. That is, ln(EWD) appears to generally decrease to an asymptote (Figure 2.5a), while both ln(TRM) and ln(LWM) have the opposite pattern of increasing to an asymptote (Appendix Figures C.1a and C.1b). LWP, on the other hand decreases to a minimum, then increases (Figure 2.5b), while ln(EWW) and MXD have the opposite trend (Appendix Figures C.1c and C.1d). Finally, age-trends for LWD and ln(LWW) (Figure 2.5c and Appendix Figure C.1e) appear to vary more from site to site, but in general, both tend to increase over the range of ages in the study.

Vargas-Hernandez et al. (1994) studied age trends of four annual ring variables (earlywood, latewood, and overall density; and latewood proportion) in 15 year-old Douglas-fir, where earlywood, latewood, and overall ring density were weighted by the area of the ring occupied by each trait, divided by the total stem cross sectional area at breast height for the trait. They found that the general form of age trends differed among traits, but were consistent over trees. Weighted latewood density increased with increasing age, while latewood proportion decreased for several years, but then began to increase; trends resembling our results for the corresponding traits of LWD (Figure 2.5a) and LWP (Figure 2.5b) respectively. Vargas-Hernandez et al., however, found weighted earlywood density to increase steadily or increase to a plateau at around age 12, while ln(EWD) was found to generally decrease to a plateau between ages 13-15 in our study (Figure 2.5c). The difference in earlywood trends appears to be due primarily to the fact that earlywood density was transformed differently in the two data sets. In particular, earlywood density is sensitive to weighting, since a large proportion of the annual ring is earlywood. To verify this, ln(EWD) data for one site was weighted in the manner employed by Vargas-Hernandez et al. and the mean of the values plotted against age. The resulting figure closely resembles the weighted earlywood density plot of Vargas-Hernandez et al. (1994) (Appendix Figure C.2).

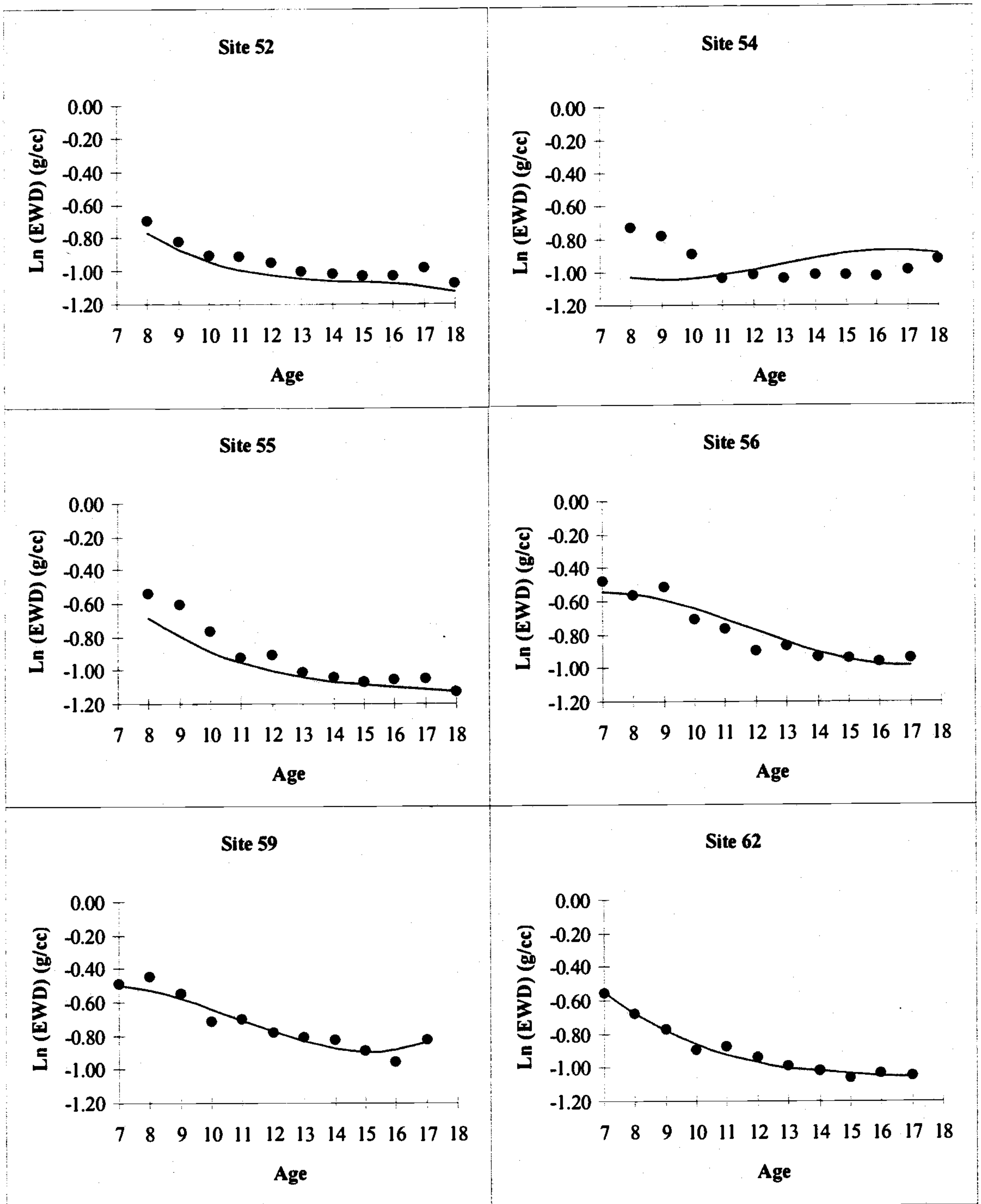

Figure 2.5a. Age-trends by site for ln[Earlywood Density (EWD)].

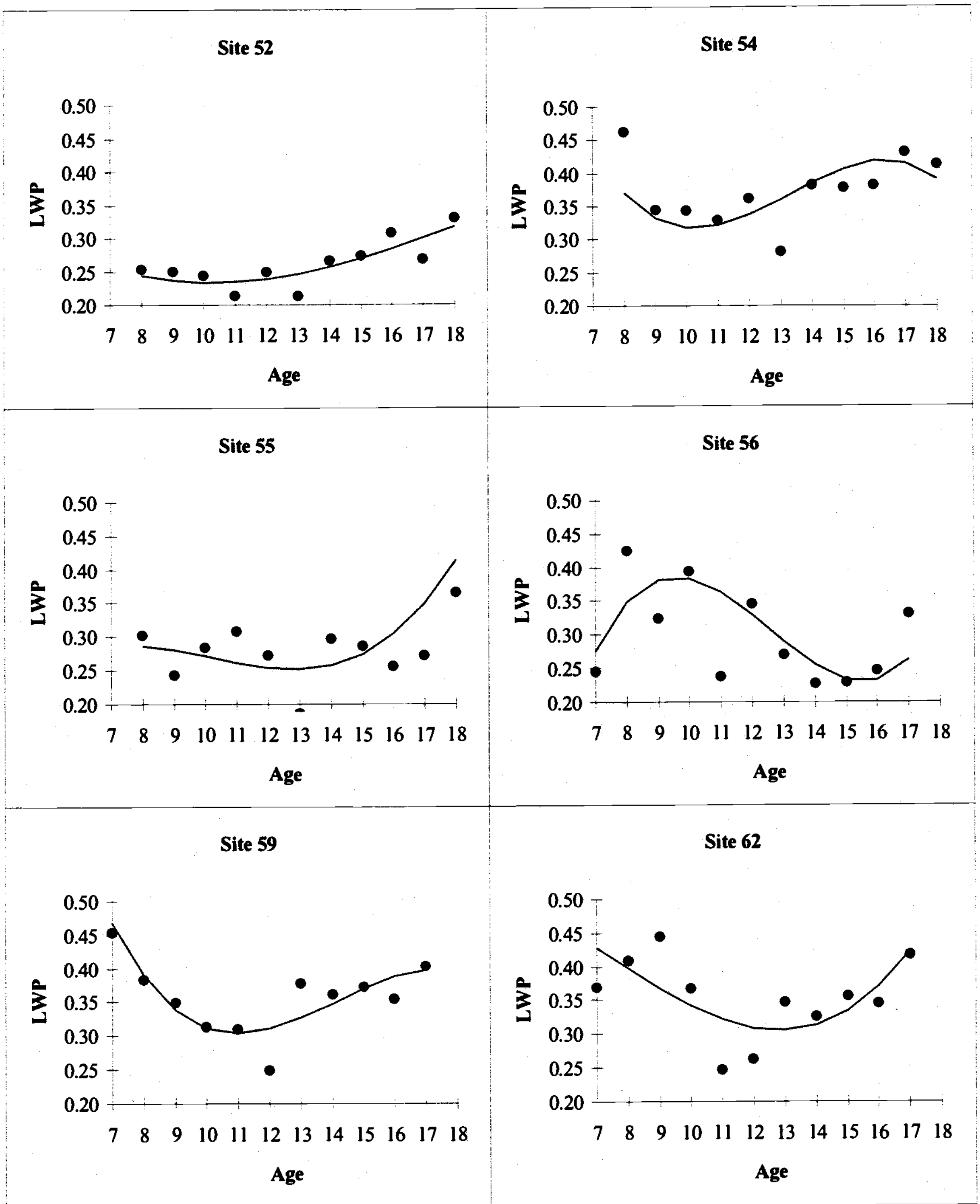

Figure 2.5b. Age-trends by site for Latewood Proportion [LWP].

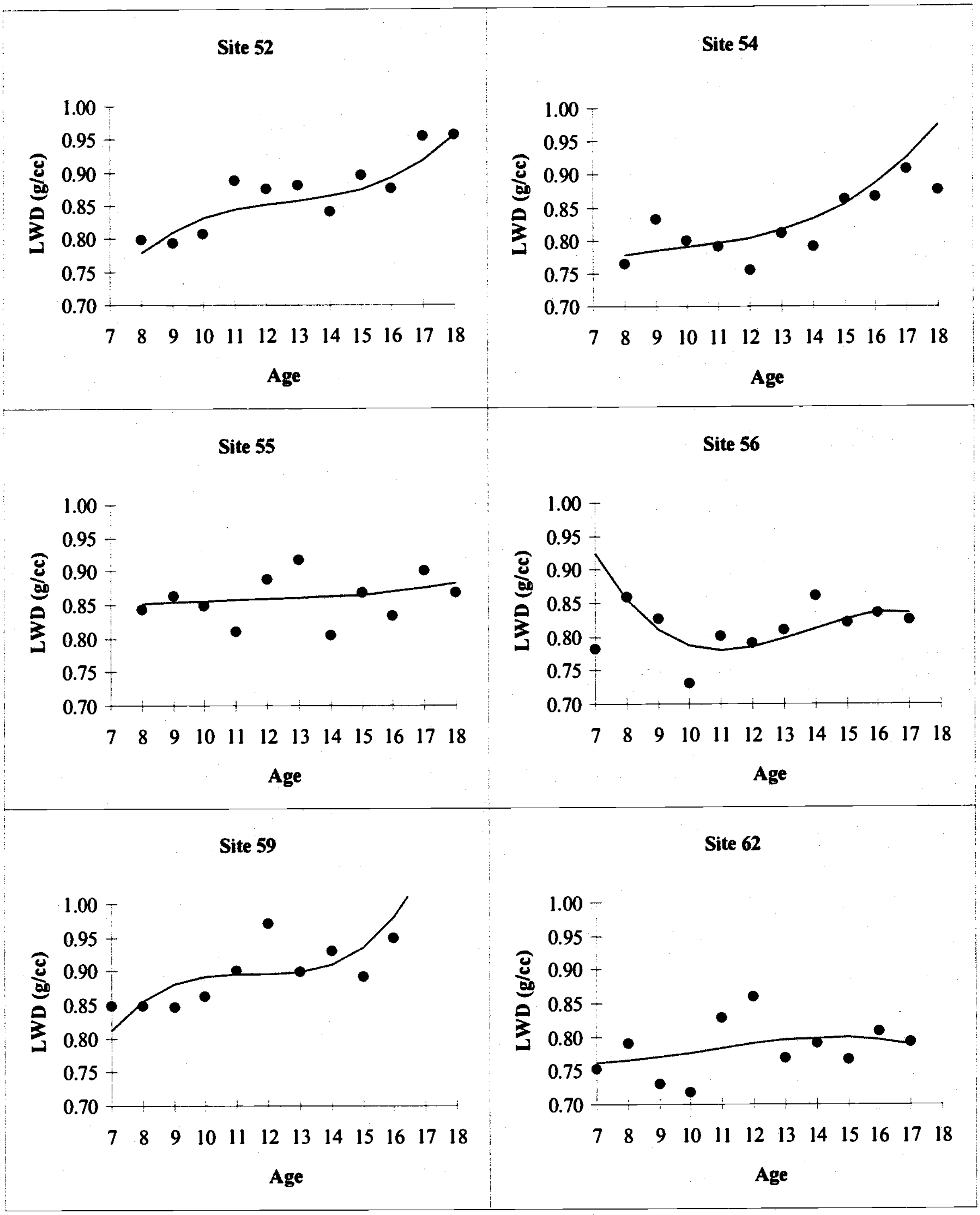

Figure 2.5c. Age-trends by site for Latewood Density [LWD].

Sensitivity of Annual Growth Ring Components to Variation in SMD

In all cases, regression models included significant terms for SMD, and for SMD x site interactions, indicating that all ring variables are sensitive to variation in SMD, but that the degree and form of sensitivity differs by site (Table 2.2). Site-to-site variation might be expected, because ring variables are likely to be sensitive to other site factors (e.g., nutrient availability, levels of intra and interspecific competition, presence of pests, etc.) that differ among the sites, but were not accounted for in the regression models. Regression analyses based on orthogonal polynomials for SMD also revealed significant site x SMD interactions for the linear trends of all ring variables. In half of the cases, linear regression terms were significant and of opposite sign in different sites, indicating lack of consistency in trends across sites (ln(LWM), MXD, ln(EWD), and ln(EWW)) (Table 2.3). In the remaining cases, however, the sign of the trend was consistent over sites when the linear regression term was significant (LWD, ln(LWW), ln(TRM), and LWP), suggesting that these ring components might be reliable indicators of drought in young Douglas-fir trees. Results for each of the eight ring variables will be presented in more detail in the next section.

Table 2.3. Slope (parameter estimates) of linear trends of eight annual growth ring components regressed on SMD, for each of six progeny test sites[a].

Annual Growth Ring Component[b]	Sites					
	Series 5			Series 6		
	52	54	55	56	59	62
LWD	**0.00144**[c]	**0.00277**	-0.00058	-0.00061	**0.00050**	-0.00023
p-value	0.0014	0.0722	0.1496	0.2147	0.0012	0.5278
ln(LWW)	**-0.01335**	-0.01464	-0.00298	**-0.00952**	**-0.00197**	**-0.00487**
p-value	0.0039	0.1114	0.3904	0.0023	0.0462	0.0394
ln(LWM)	**-0.00347**	**0.00428**	0.00172	**0.00268**	0.00034	0.00059
p-value	0.0097	0.0617	0.1081	0.0224	0.6418	0.5988
ln(TRM)	**-0.01105**	0.00221	**-0.00846**	-0.00298	**-0.00117**	**-0.00456**
p-value	0.0006	0.7543	0.0002	0.1442	0.0524	0.0026
MXD	**-0.00824**	**0.01527**	**-0.00482**	**-0.00159**	**0.00034**	-0.00021
p-value	0.0003	0.0969	0.0050	0.0836	0.0569	0.7472
LWP	**-0.00376**	**-0.00407**	-0.00087	**-0.00284**	**-0.00058**	**-0.00219**
p-value	0.0018	0.0820	0.3299	0.0003	0.0237	0.0003
ln(EWD)	-0.00714	**-0.05766**	**-0.00635**	**0.00284**	**-0.00108**	0.00116
p-value	0.1121	0.0001	0.0378	0.0365	0.0003	0.243
ln(EWW)	0.00916	0.05052	**-0.02211**	**0.00651**	**0.00132**	0.00390
p-value	0.4141	0.1415	0.0061	0.0695	0.0913	0.1334

[a] Based on regression analyses using orthogonal polynomials (See text).
[b] See table 2.2.
[c] Numbers in **bold** are significant at $p \leq 0.10$.

Ring Variables with Consistent Associations with SMD Over Sites

Latewood Width and Proportion

Ln(LWW) and LWP had relationships with SMD that were fairly consistent across sites (Figures 2.6 and 2.7). The curves are the predicted site-means for these ring variables at age 13 (mid age for the data in the study), for the range of SMD values experienced on each site. Individual data points were derived by subdividing the SMD values into five or six relatively equal classes, and estimating the value of the ring variable for each class at age 13 (i.e., assuming no *a priori* relationship between the ring variable and SMD). The general fit of the regression curves for these two ring components seems quite good, although there is considerable variation of the individual data points about the regression line for site 56. Ln(LWW) and LWP have similar relationships with SMD across the sites. This similarity is not unexpected, because the two traits are linked mathematically (LWP = LWW/Total ring width) and is corroborated by the modest correlation between the two variables ($r = 0.54$) (Appendix Table E.1). Regression model building resulted in the same form of the final model for both variables, with a significant cubic term for SMD, but only a quadratic SMD x site interaction term (Table 2.2).

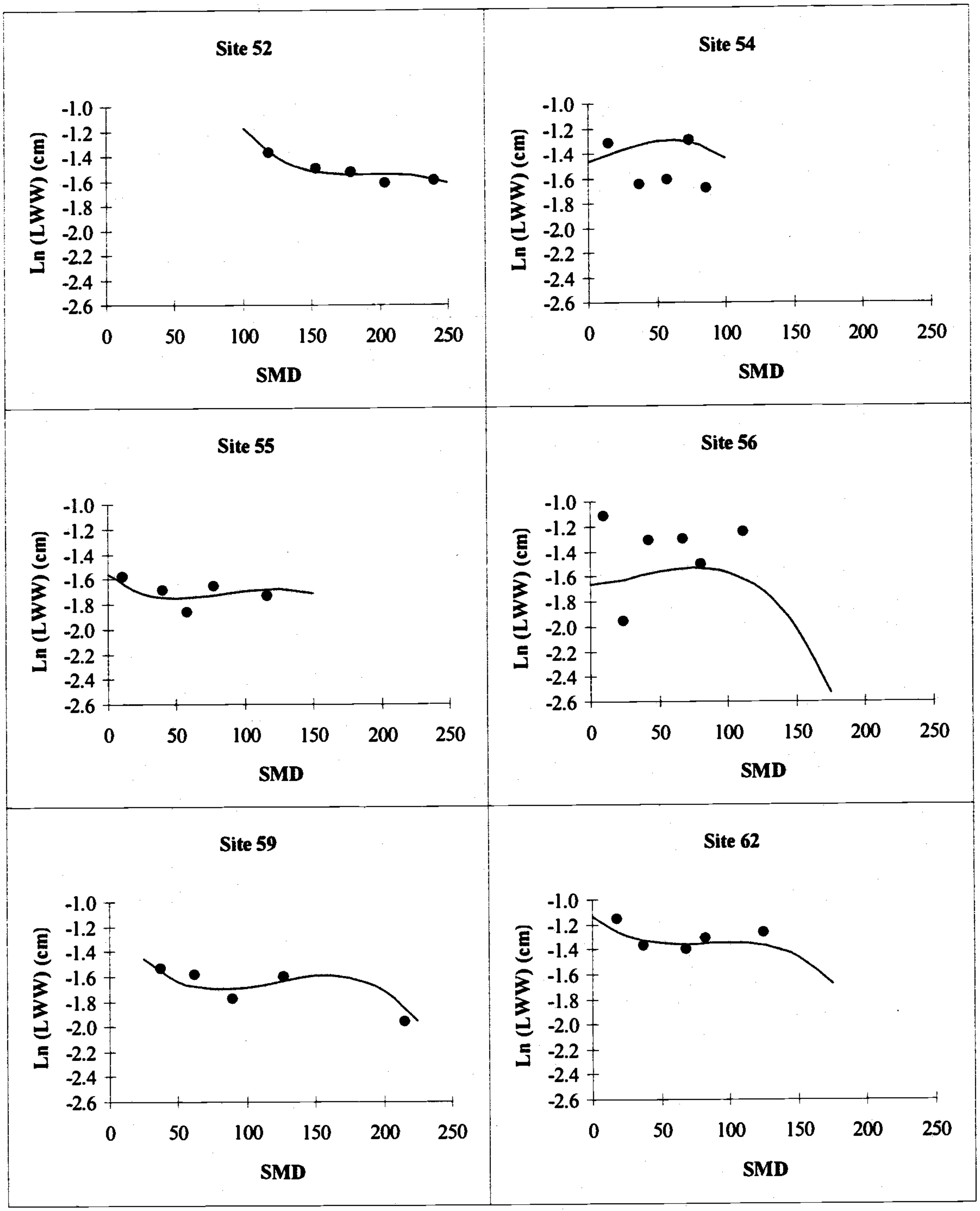

Figure 2.6. Regression of ln[Latewood Width (LWW)] on Soil Moisture Deficit (SMD) for each of six test sites.

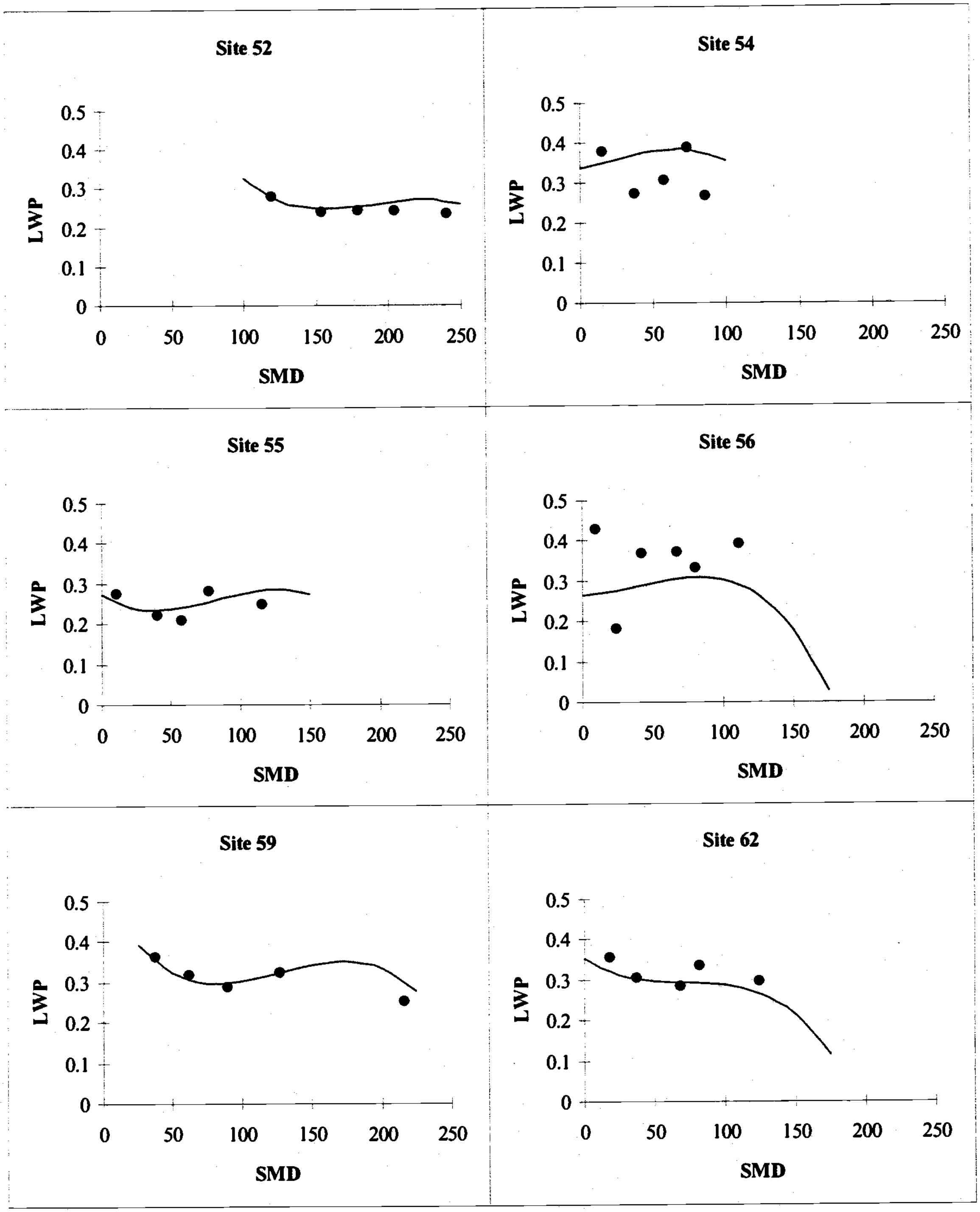

Figure 2.7. Regression of Latewood Proportion (LWP) on Soil Moisture Deficit (SMD) for each of six test sites.

Although the curves were significantly heterogeneous over sites, both ring components seem to generally decrease with increasing SMD. This is confirmed by the linear regression term from the orthogonal polynomial analyses of both ring variables, which were negative for all sites and significant ($p \leq 0.10$) in most cases (Table 2.3). Both LWW and LWP are expected to decrease with increasing SMD if moisture stress occurs in the latter part of the growing season when latewood is produced.

Our results agree with earlier studies of the affects of moisture deficit on latewood width. Woods and Debrunner (1970) found that latewood diameter in loblolly pine decreased with increasing number of drought days in August and September, and Robertson et al. (1990) reported that LWW in Douglas-fir was strongly depressed in years with high water deficits. The effect of drought on LWP has been investigated in numerous studies, but without consistent results. Zahner (1962) studied growth and density of wood produced by 5 year-old loblolly pine trees grown under well watered and imposed drought treatments, and reported that the percentage of latewood was less in the well-watered treatment. Zahner et al. (1964) studied the effect of irrigation and imposed drought on 20-year old red pine and found that although the total growth of the irrigated trees was much greater that that of drought treated trees, there were no consistent patterns in LWP. Cregg et al. (1988) studied the effect of stand density and climate on growth and wood quality of 10 year-old loblolly pine. In two years following thinning they found that LWP for all treatments was 9.5% lower in the second year (a warm, dry year) compared with the first year (a relatively wet year). Robertson et al. (1990) found a strong positive effect of annual water deficit on LWP in Douglas-fir on their wettest site. Kennedy (1961) reported that in older (65-81 year-old) Douglas-fir, LWP was negatively correlated with the length of the period of earlywood formation and positively correlated with the length of latewood formation. As a consequence, climatic factors that effect the length of the growing season, and the timing of the transition from earlywood to latewood, have effects on LWP. He suggests that low precipitation, high temperatures, and long periods of sunshine (all of which would lead to increased SMD) promote high LWP. On the sites in this study, there is considerable variation in monthly SMD (Appendix Figure B.1) across years, which may influence the timing of the transition from earlywood to latewood, thus affecting LWP. These examples seem to illustrate the fact that latewood percentage is greatly influenced by the level of moisture stress that the tree is experiencing. However, the effect seems to vary quite dramatically depending on what part of the growing season the trees experience this moisture stress, and what the climatic conditions are like during the rest of the season.

Latewood Density

Regression analysis revealed that LWD has a quadratic relationship with SMD (Table 2.2), but that the form of this relationship is of two different types depending on site (Figure 2.8). In four of the sites (52, 55, 56, and 62), LWD first generally increases with increasing SMD, but then reaches a maximum and begins to decrease. The maximum value seems to vary with site, in the range of SMD from 75 to 175. This variation could be due to estimation error (e.g., small number of trees per site, inadequacy of climate modeling, etc.) or unknown site factors other than SMD influencing LWD. In the remaining two sites (54 and 59) there appears to be a steady increase in LWD with increasing SMD. Linear trends were either significant with a positive slope (sites 52, 54, and 59), or not significant (Table 2.3). In their study of older Douglas-fir trees (70 years-old), Robertson et al. (1990) also found that the relationship between LWD and SMD varied over sites. On their wet and moderately dry sites, they found that LWD increased linearly with summer water deficit, although the slope was shallower on the moderate site. On the driest of their three sites, however, LWD increased during mildly moisture stressed years, but declined during highly moisture stressed years, similar to our results from several sites. Wood density depends on cell diameter as well as cell wall thickness, as both smaller diameter cells, or thicker cell walls would both result in higher density. Preliminary results from a small side study on a limited number of samples indicates that a decrease in latewood cell lumen diameter is likely the reason for the increase in density. No significant differences were detected in cell double wall thickness, however the sample size was severely limited. It is possible that at high SMD levels, cell production and cell wall thickening are impeded, resulting in thinner cell walls, leading to a reduction in density as was observed on several sites. Another possible explanation for the decrease in LWD at high levels of SMD is that if cell production ceases prematurely due to high levels of moisture stress, the highest density latewood may never be produced, resulting in a lower mean LWD. These hypotheses, however, could not be tested with our results, and should be studied in more detail in the future.

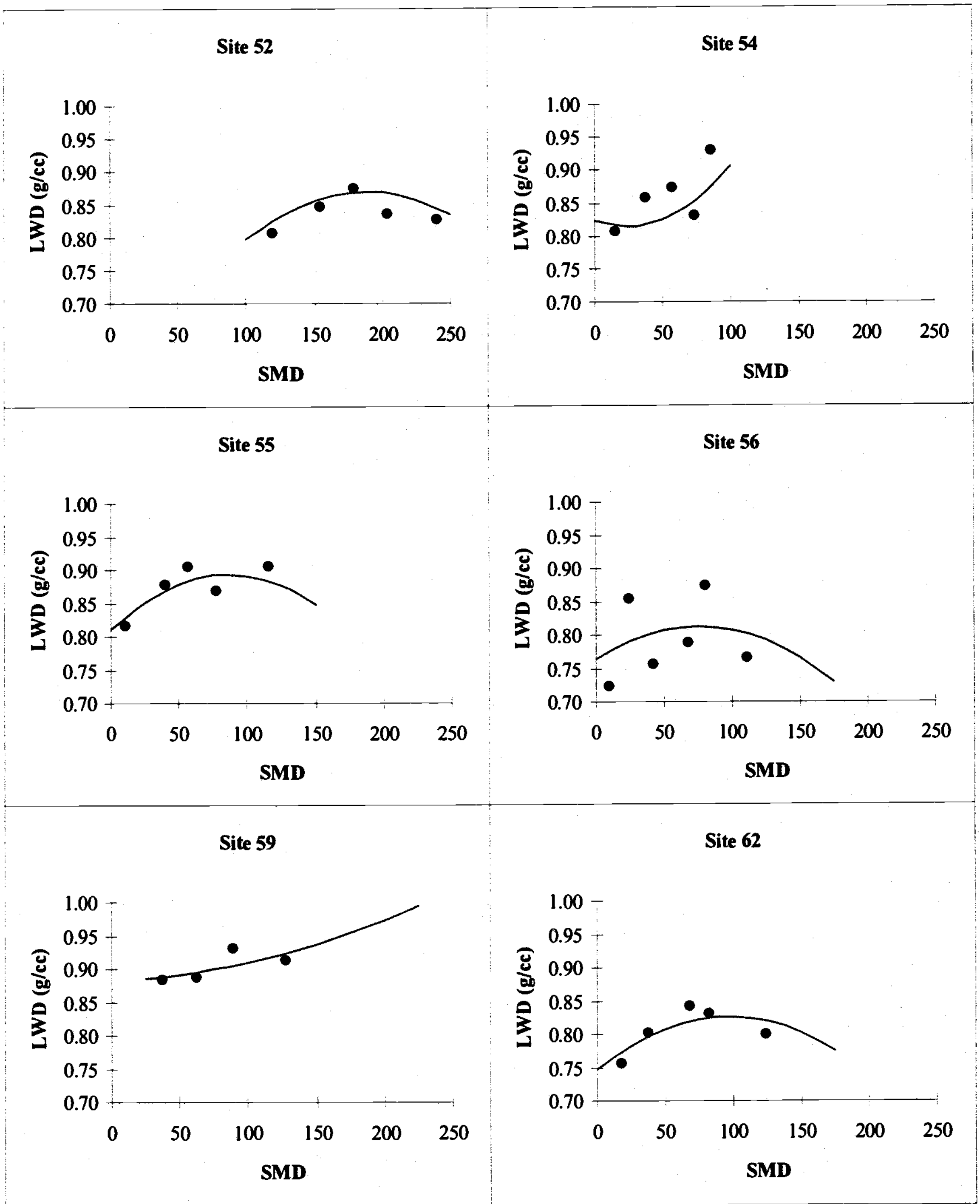

Figure 2.8. Regression of Latewood Density (LWD) on Soil Moisture Deficit (SMD) for each of six test sites.

Total Ring Mass

The best fit of the association of ln(TRM) with SMD was a cubic model (Table 2.2). In all cases but one (site 54), ln(TRM) decreased with increasing SMD, but in a pattern where the decrease occurred primarily at low and higher values of SMD, with little change in ln(TRM) at mid-range values of SMD (Figure 2.9). The exception (site 54) seems to be the primary reason that significant heterogeneity in patterns was observed over sites. Indeed, inspection of the linear trends (Table 2.3), shows that site 54 was the only site where a positive linear trend was found, but the regression coefficient is not significantly different from zero. In four of the five remaining cases, the linear trend was significant, with a negative slope. TRM appears to be primarily a function of ring width rather than ring density, since the correlations of ln(TRM) with ln(EWW) and ln(LWW) were both moderately strong and positive ($r = 0.64$ and 0.60 respectively), but were weak ($r = 0.22$) or negative ($r = -0.37$) with LWD and ln(EWD) respectively (Appendix Table E.1). Thus, reduction in ln(TRM) with increasing SMD appears to be primarily due to overall ring growth being impeded at higher SMDs. A negative association between ring mass and SMD was also observed in older (70 year-old) Douglas-fir by Robertson et al. (1990), who reported that annual water deficit (SMD) had a strong influence on ring weight (mass) on all sites.

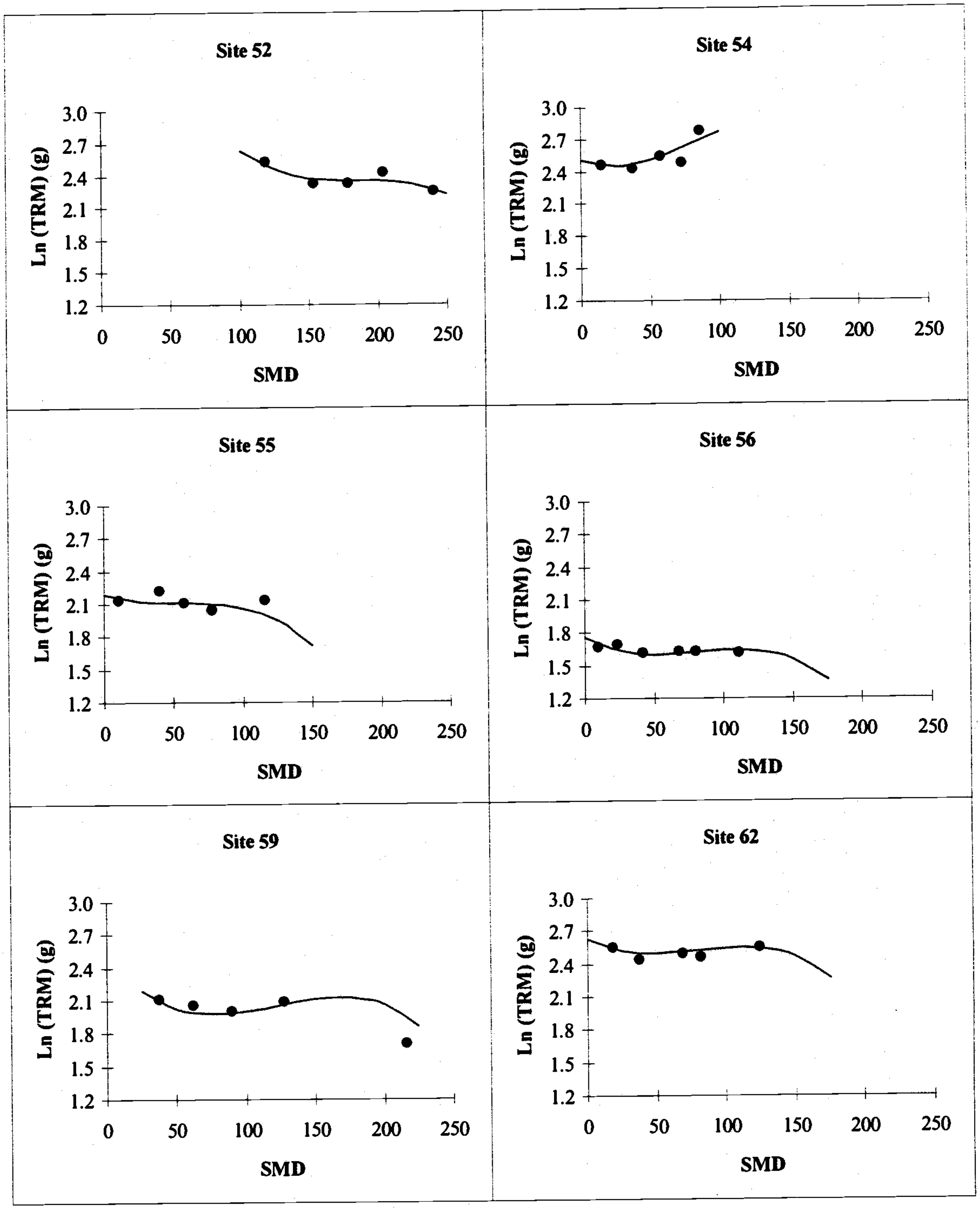

Figure 2.9. Regression of ln[Total Ring Mass (TRM)] on Soil Moisture Deficit (SMD) for each of six test sites.

Ring Variables with Inconsistent Associations with SMD Over Sites [ln(LWM), MXD, ln(EWD), and ln(EWW)]

Like the ring variables consistently associated with SMD across test sites, the four ring variables with inconsistent trends were also best fit with quadratic or cubic terms for SMD (Table 2.2). LWM is a function of both the density and width of the latewood, which are influenced in opposite ways by increasing SMD (Table 2.3). Thus, it is perhaps, not surprising that linear trends in ln (LWM) were not consistent across sites (Table 2.3). Presumably the direction of the slope of the trend in ln(LWM) is determined by whether width or density of the latewood has the dominating influence on LWM on a particular site.

One might expect MXD to be positively associated with SMD, because the most dense wood is produced during mid to late summer when sites are driest (Appendix Figure B.2). Indeed, MXD has been found to increase with increasing summer temperatures and decreasing precipitation in a number of tree species (Conkey, 1979, Kienast et al., 1987). In this study, however, significant linear trends in MXD with SMD were positive in only two sites, and negative in three (Table 2.3). One possible reason for inconsistent linear trends in MXD is that it relies on the single measurement with the highest density, whereas all other growth ring variables are integrated over many X-ray measurement points. This makes MXD particularly sensitive to sampling error, as an anomaly in the wood at a particular spot could result in an abnormally high density value, that may not be related to the influence of climate. Another possible explanation is that the response of MXD to SMD is similar to that of LWD. MXD may be increased under mild and moderate levels of SMD, but is actually decreased if growth ceases earlier at higher levels of SMD due to moisture stress. If moisture stress becomes limiting to growth, the highest density latewood, which is produced at the end of the growing season, may never be produced, resulting in lower MXD values. Indeed, the regressions curves of LWD and MXD have similar shapes when compared (Figure 2.8 and Appendix Figure D.1a), and these two variables have a relatively high correlation ($r = 0.66$) (Appendix Table E.1).

Earlier results in Douglas-fir (Robertson et al., 1990) and loblolly pine (Woods and Debrunner, 1970) indicate that earlywood density (EWD) and width (EWW) are insensitive to annual SMD, because earlywood is formed primarily in the period before significant summer drought occurs (spring and early summer) (Appendix Figure B.1 and B.2). Significant linear associations of ln(EWD) and ln(EWW) with SMD, however, were found in this study (Table 2.3). Clearly these earlywood variables are not insensitive to annual SMD, however, the linear trends for both of these variables were inconsistent, with slopes of opposite sign on different sites. Robertson et al. (1990) found that both earlywood width and density are influenced by growth conditions of the previous year. These residual effects, which are probably only partly accounted for in the statistical analysis, in addition to the timing of the period of earlywood production in contrast to the period when SMD occurs are the likely causes for the inconsistent relationships of earlywood width and density with SMD.

A number of additional factors may have contributed to the lack of consistency in the association of these four ring variables with SMD, and in the varying form and magnitude of the responses of the other four ring variables across sites. Probably the most important contributing factor is that the study sites covered different ranges in total annual SMD (Appendix Table B.1, Figures 2.6 - 2.9, Appendix Figures D.1a - D.1d). Four of the sites experienced only low levels of SMD (54, 55, 56, and 62), one experienced the entire range from low to high (59), and one experienced only higher levels of SMD (52). It might be expected that the trees growing on sites experiencing different levels of SMD would respond somewhat differently to changes in SMD.

Another important factor contributing to inconsistency in trends across sites is experimental error. There is error due to the relatively low number of individuals that were sampled on each site (16), and also several sources of modeling error. The modeling is constrained by the use of a climate model, in which there is an inherent level of error associated with the use of mathematical relationships to describe biological processes (such as evapotranspiration). In the case of this study, this is exacerbated by the fact that the basic meteorological data used in the model may not be accurate, as it was interpolated from weather stations that experienced somewhat different weather patterns than the actual study sites themselves. Yet another complicating factor is microsite variation. A single SMD value was calculated from the water balance model for each site. Nevertheless, variation in soil depths and types, and the occurrence of rocky outcroppings, decaying organic matter, seeps, etc. across sites, can lead to spatial variation in SMD. In response to this, it should be noted that the sampling scheme was designed to minimize this variation, and in addition, these sites were chosen by the British Columbia Ministry of Forests as progeny test sites partially because they were generally free of major microsite variation. It is impossible, however, to completely eliminate all microsite variation on even the best of sites.

Another source of modeling error is the regression model itself. In this analysis, age x site x SMD interactions, and interactions of age and SMD with series and families within series were not included in the model, in order to prevent it from becoming unwieldy, however, these interactions may be important. For example, the response of ring variables to SMD may differ with the age of the tree when the ring was produced. In particular, the earliest rings may be more susceptible to influences by microsite variation and by non-site factors (e.g., size of seedling at planting, root biomass, etc.). Thus, before full capture of the site and the onset of tree-to-tree competition, annual ring development may be more a factor of inherent growth potential, and less influenced by yearly weather conditions, especially relatively modest differences in soil moisture availability.

Conclusions

Components of annual growth rings in young, plantation-grown Douglas-fir are strongly affected by age trends. In addition, these components seem to be sensitive to drought, as indicated by their response to varying levels of annual SMD. During the spring, soil water on Vancouver Island and coastal mainland British Columbia is fully recharged from winter rains. As the growing season progresses, the soil gradually dries out, and in mid to late summer some sites may experience moderate to severe SMD. Although earlywood components appeared to be sensitive to SMD, their associations with SMD were not consistent across sites. Earlywood formation occurs early in the growing season, and thus, is probably more susceptible to the timing of the onset of drought which varies by site and year. Latewood is formed later in the growing season, when at least some SMD is likely to be experienced. Thus, associations of latewood components with the magnitude of drought, appears to be more consistent across sites. Our results indicate that measuring the response of latewood components of annual growth rings to soil moisture deficit may be a useful and relatively simple way to non-destructively assess trees for sensitivity to drought. Four annual growth ring components; latewood density, latewood proportion, latewood width, and total ring mass, are sensitive in a consistent and predictable manner, and thus appear to be the most promising for use in screening families for differences in drought response. If families are found to differ in drought sensitivity as revealed by growth ring analysis, this technique might prove very useful for screening improved families for drought hardiness in breeding programs.

CHAPTER 3 - EFFECT OF SOIL MOISTURE DEFICIT ON LATEWOOD RADIAL CELL LUMEN DIAMETER AND DOUBLE WALL THICKNESS

Introduction

One of the responses to SMD observed in this study, is that LWD increases with increasing soil moisture deficit. An interesting question is the basis of this increased density at the cellular level. Density increases with decreased cell size and increased cell wall thickness, so changes in density could result from a change in either or both of these parameters. In order to evaluate the morphological basis of increased LWD, radial cell lumen diameters and double wall thickness of cells in the latewood section of annual growth rings from wet and dry years were compared.

Site Selection, Sampling, and Measurement

Latewood was sampled from an individual core of one tree from each of the eight different families on a single site (55) and radial cell lumen diameter and cell double wall thickness measured (Figures 3.1 and 3.2). Radial cell lumen diameter measurements from one core were inadvertently lost, so cell diameter data were available from only seven cores. All eight cores were used in the analysis of cell double wall thickness. To be ablé to distinguish morphological differences at the cellular level, it was important to choose a site that experienced very different SMD levels on different years. It was also important that these years not be consecutive, in order to help eliminate possible confounding due to residual effects from the previous growing season (lag effects). Site 55 was chosen because it had a dry year (1989) (SMD = 105.9 mm) and a wet year (1993) (SMD = 0.0 mm) (Appendix Table B.1) separated by three years in-between.

Radial sections were displayed at a 400x magnification (40x objective with 10x eyepiece) on a computer screen in black and white using a microscope with a video interface, and measurements made by manually drawing lines on the computer screen (Figure 3.2), using a line drawing tool available in the public domain NIH Image computer program (developed by the U. S. National Institutes of Health and available on the Internet by anonymous FTP from zippy.minh.nih.gov or on floppy disk from National Technical Information Service, Springfield, Virginia, part number PB95-5001 95 GEI). The computer measured the length of these lines in pixels, which could then be converted to microns by calibration with an objective micrometer slide. Radial section slides were prepared by excising a section of the latewood with a razor blade, suspending the sample in distilled water, and covering with a cover slip. Measurements were made on as many cells that fit in a single tangential row of cells in the 0.22 mm x 0.18 mm field of view (approximately eight). Measurements of cell lumen diameter and cell double wall thickness on both radial sides of each of the cells were recorded from three fields of view sampled for each year. In order to measure “normal” latewood cells, and not anomalous “flattened” cells that form at the growth ring boundary, measured tangential rows were approximately eight cell-rows prior to the growth ring boundary (Figure 3.1).

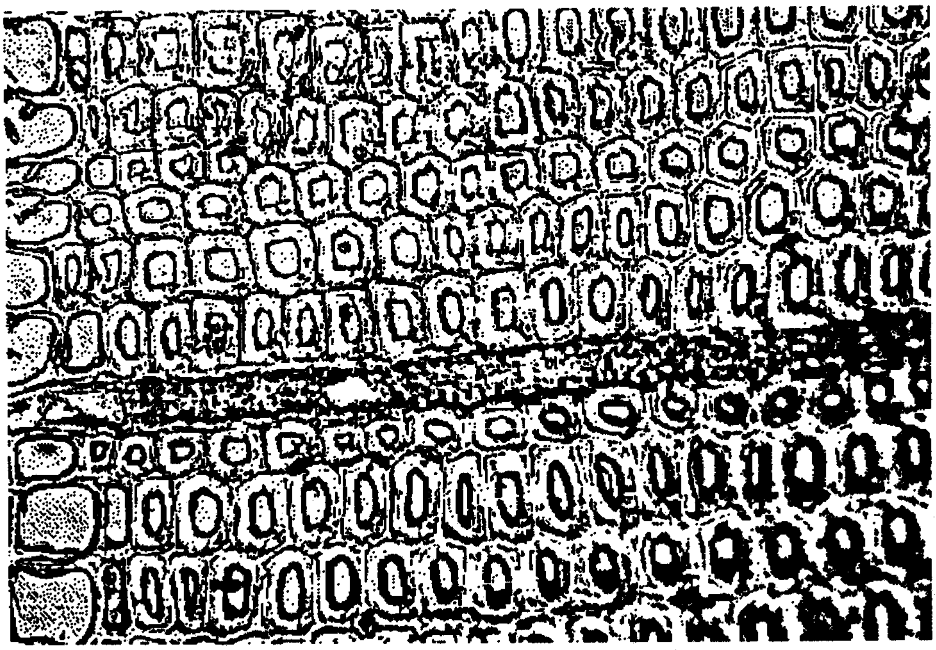

Figure 3.1. Microscope image of latewood cells on which radial cell lumen diameter and double wall thickness measurements were made. (The ring boundary between the latewood of one year and the earlywood of the next year can be seen on the left side of the image. The large cells on the left border are the first row of earlywood cells of the next annual growth ring.)

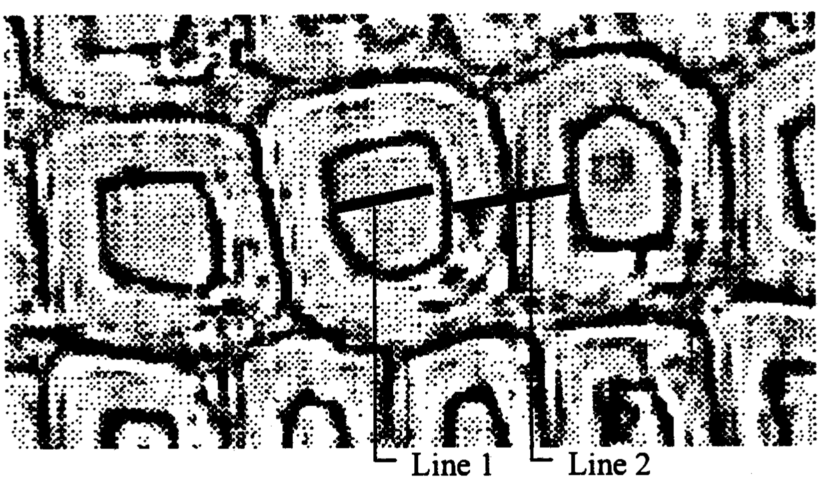

Figure 3.2 Example of radial cell lumen diameter and double wall thickness measurement. (Line 1 measures cell lumen diameter, Line 2 measures double wall thickness.)

Analysis, Results, and Discussion

One-tailed paired t-tests (SAS PROC MEANS, SAS Institute Inc., 1989a) were performed to test the null hypotheses (H_o) that the mean differences of radial cell lumen diameter and cell double wall thickness between a dry year (1989) and a wet year (1993) are equal to zero (H_a: radial cell lumen diameter is smaller and cell double wall thickness is larger in a dry year). The difference between means (dry year - wet year) of cell lumen diameter was -0.1799 μm, and strong evidence was found to reject the null hypothesis that the mean difference in radial cell lumen diameter was equal to zero ($p = 0.0046$). The difference in means of cell double wall thickness was 0.0187 μm, and no evidence was found to reject the null hypothesis that the mean difference in cell double wall thickness is equal to zero ($p = 0.4400$). Although mean cell lumen diameter was significantly less in the dry year than in the wet year, it was unclear whether the results were confounded by age effects, so additional measurements were made on the intervening years (1990, 1991, and 1992), for three of the seven trees. Repeated measures regression analyses with a first order autoregressive covariance structure were performed for both cell lumen diameter and cell double wall thickness on age for all five years from these three trees, to test the null hypothesis that the slopes of the regression lines were equal to zero. On this site, LWD appears to generally increase with age, although the relationship is weak (Figure 2.5c). Thus, on the effect of age alone, we would expect cell diameter to be lower in 1993 than in 1989, and the slope to be negative. The regression of cell lumen diameter on age provided strong evidence to reject the null hypothesis ($p = 0.0190$), with a positive linear regression coefficient estimated, opposite to what was expected on the basis of age, but consistent with the general decrease in SMD over the five year period. The regression of cell double wall thickness on age failed to detect a slope significantly different from zero ($p = 0.4706$). These results indicate that while there may be an age effect on cell lumen diameter, it appears to be opposite to the effect of SMD, supporting our alternative hypothesis that cell lumen diameter is smaller in dry years. These results indicate that at the cellular level, it is likely that reduced cell lumen diameter is the morphological reason for an increase in LWD during dry years. This investigation of cell morphology, however, had a very limited sample size; the results need to be verified by further investigation.

CHAPTER 4 - THESIS CONCLUSIONS

Overall Conclusions

This study investigated the effects of ring age and SMD on eight components of annual growth ring variables. In addition, a small auxiliary investigation was used to examine the morphological basis at the cellular level for the observed positive association between latewood density and SMD. It was found that:

- Age-trends and SMD effects on annual growth ring components are adequately modeled in a repeated measures type regression analysis by polynomial parameters up to and including cubic terms.

- Components of annual growth rings are strongly influenced by age trends, although significant age x site within series interactions indicated the age-trends differed somewhat over sites.

- After accounting for age-trends, all of the annual growth ring components investigated appear to be sensitive to drought (SMD), but not all components yielded the expected responses and were consistent across sites.

- The best fitting relationships of the growth ring variables with both age and SMD were often complex, involving quadratic and cubic terms, and were significantly heterogeneous across sites, which made interpretation of the trends somewhat difficult.

- Linear trends in the ring variables over SMD were consistent across sites (i.e., slopes had the same sign, when significant) for latewood density, latewood proportion, ln(latewood width), and ln(total ring mass). In addition, the sign of the slopes agreed with the expected response of trees to limited moisture availability. These components appear to be reliable indicators of drought sensitivity, and thus may be quite useful for assessing response of young Douglas-fir families to drought.

- Linear trends for the four remaining ring variables, maximum density, ln(latewood mass), ln(earlywood density), and ln(earlywood width) were not consistent across sites with significant trends.

- Increased latewood density with increasing SMD seems to be primarily the result of reduced cell size and not increased cell wall thickness.

Recommendations for future research

Family Variation in Drought Response

This project is the first part of an intended two part study. The first part was to determine whether annual growth ring variables in young trees are sensitive to drought. If one or more growth ring variables were found to be sensitive in a manner that is reasonable and predictable, the second part was to determine if there are genetic (family) differences in sensitivity and response to drought. If family differences are found, ring variables sensitive to variation in SMD might prove to be useful for screening families for drought hardiness; to determine which families to deploy in drought-prone sites, or to use in breeding drought-hardy varieties. Both the number of families (16 different families, eight from each of two sets of crosses), and individuals per family (two trees per family on each of four sites) were limited in this study. This sampling scheme was intended only to ensure a variety of genotypes in the sample, and not to allow testing for family differences. The results of this study are sufficiently promising to warrant continuation with the second part of the study. Latewood components of annual growth rings are the most promising for further investigation; more specifically, latewood density, latewood width, latewood proportion, and total ring mass, as they seem to give predictable and reliable results. For the second stage of the study, I recommended sampling trees from site 59. This site has the second highest mean annual SMD (88.76), and the largest range of SMD (32.8 - 215.6) (Appendix Table B.1) of the eight sites originally sampled (Figure 2.4). In addition, the tests of the linear trends for the four most promising growth ring variables were all significant on this site (Table 2.3). Thirty-nine families currently growing in raised nursery beds for a PNWTIC funded study investigating seedling drought hardiness are also found on this site, making it possible to relate drought response and drought hardiness in sapling-age trees to drought hardiness at the seedling level. My recommendation was followed, and increment cores (one from each tree) were collected from site 59 in October of 1997. All trees from each of the 39 families were sampled, giving a total of 475 cores (mean 12.2 per family).

Morphological Explanation for Increased Density

It would be useful to understand not only how trees respond in situations where moisture is limited, but also what morphological changes occur at the cellular level in response to drought. The auxiliary investigation described in chapter 3 was an attempt to try to understand why LWD increases as SMD increases. Although the hypotheses being tested in this investigation were interesting, the amount of time available for sampling was limited, and therefore, only a very small sub-sample of the materials used in the main study were examined. With the limited sample size available, a significant difference in latewood cell lumen diameter between wet and dry years was detected, however, these results need to be confirmed through further investigation, with a more comprehensive sample. In order to understand the morphological reasons for the response observed, a larger sample of both the number of cores (trees), and the number of rings on each core is necessary. It would still be useful to study cell lumen diameter and double wall thickness within the latewood, but another measurement that might be informative is the ratio of cell lumen area to cell wall area in the field of view of the microscope. If an increase in density is due to a decrease in cell lumen diameter as opposed to an increase in cell wall thickness, the cell lumen area:cell wall area ratio would decrease during dry years. A change in this ratio might be linked to the weather conditions at the time the cells were produced. These measurements could be used to test the null hypotheses that the effect of SMD on radial cell lumen diameter, cell double wall thickness, and cell lumen area:cell wall area ratio is equal to zero. This could be tested using a repeated measures type regression analysis similar to the one used in the main study of this thesis. Age-trends should be carefully investigated first, and if any are found, they should be accounted for in the regression, as was done for the response variables in the main study, this way, the true effect of SMD on cell morphology can be determined

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APPENDICES

APPENDICES

Data and other information supporting the methods and results discussed in this thesis are presented in the appendices that follow. Appendix A describes the methods used to remove extractives from the increment cores, x-ray densitometry procedures, and how density values are derived from the densitometer output. This appendix also includes a table of site means and ranges for all eight annual growth ring variables analyzed in the study (Appendix Table A.1). Appendix B contains a table of estimated yearly total growing season soil moisture deficits for the eight sites sampled (Appendix Table B.1), as well as yearly climate data used in the water balance model to calculate the soil moisture deficit for each of the sites (Appendix Tables B.2a - B.2h). In addition, Appendix B contains charts of the estimated monthly SMD values for the eleven years of the study for site 62 (Appendix Figure B.1) and of the mean estimated monthly SMD values for each of the six sites in the study (Appendix Figure B.2). Appendix C contains charts of the age-trends for each of the five annual growth ring variables (MXD, ln(TRM), ln(LWW), ln(LWM), and ln(EWW)) not presented in chapter 2 (Appendix Figures C.1a - C.1e), and a chart of EWD weighted using the method of Vargas-Hernandez et al. (1994) on age (Appendix Figure C.2). Appendix D contains charts of the curves described by the final regression models for the four annual growth ring variables with "inconsistent" results (MXD, ln(LWM), ln(EWD), and ln(EWW) (Appendix Figures D.1a - D.1d). Appendix E contains a correlation matrix for the eight annual growth ring variables analyzed in the study (Appendix Table

E.1). Appendix F contains examples of the SAS code used to perform the regression analyses for one response variable (LWD), and the SAS output resulting from this analysis.

APPENDIX A: Core Extraction, X-ray Densitometry, and Growth Ring Component Measurement

Core Extraction

Prior to X-ray densitometry, the increment cores, sawn to approximately 1.5mm thick were treated to remove extractives such as resin that might be present in the wood. It is thought that resins and other extractives alter the attenuation of X-rays, resulting in inaccurate measurements from the X-ray densitometer. The resins and extractives were removed from the wood by submerging the samples in a near boiling 2:1 mixture of 95% ethyl alcohol:toluene (Park et al. 1989). A beaker with the solvent and the cores was placed in a fume hood on a hot plate and kept near boiling. The solution was changed every two hours with a fresh mixture, increasing the ethyl alcohol:toluene ratio, until the solution was almost all ethyl alcohol. After six total hours of chemical extraction, the cores were removed from the beakers, placed on paper towels on a flat surface in the fume hood with a weight on top of them (a heavy book), and allowed to dry.

X-Ray Densitometry

The samples were positioned in the X-ray beam on an x-y table that is linked via a Windows based PC to the densitometer. The table moved the length of the sample through an X-ray beam at 100 micrometer increments, recording the time it takes for a specified number of x-ray photons (10,000) to pass through the sample. Parallel waves of radiation passing through a homogeneous medium is attenuated according to Beer's Law (Jones, 1992), and this equation is used to determine the relative density of the sample.

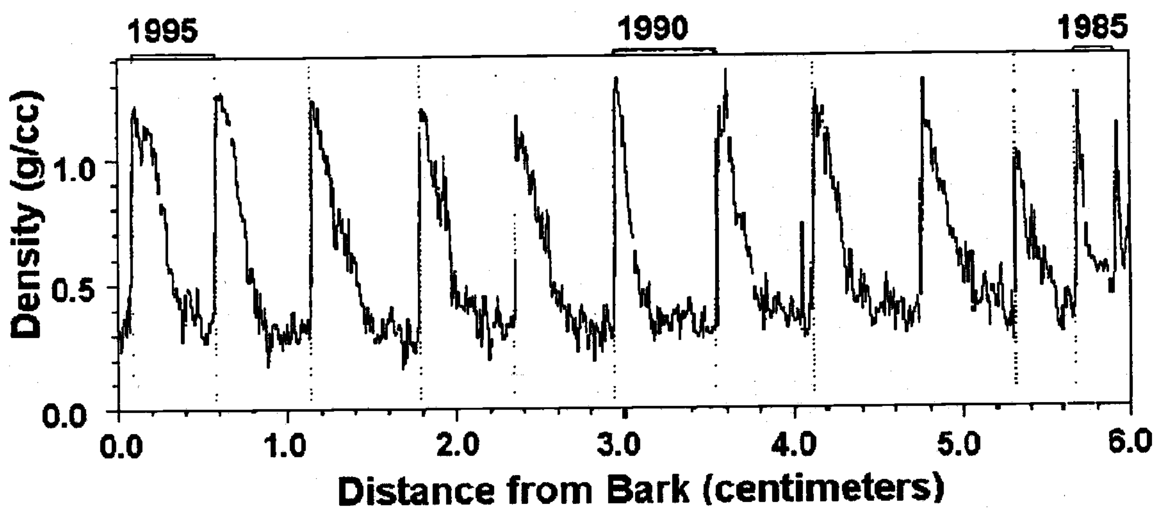
$$D = [- \ln (I/I_0)] / (ZK)$$

Where D = density
- I = flux density of X-rays received through air
- I_0 = flux density of X-rays received through sample
- Z = thickness of the sample
- K = mass attenuation coefficient

The mass attenuation coefficient, K, is dependent on the material and moisture content of the sample. The moisture content of the cores fluctuated slightly depending on relative humidity. The mass of each core before and after X-raying, as well as their oven dry mass was recorded, so that average moisture content, and the change in moisture content from before and after X-raying could be calculated for each core. Moisture contents ranged from 8.5% to 10.7% with a mean of 9.9%. Although the moisture content of the samples did fluctuate, the small magnitude of the changes had only a trivial effect on density. Thus, for the purposes of this study, where only relative density values are necessary, the attenuation coefficient was ignored in the calculation of density.

Growth Ring Component Measurement

The calculated density values were fed into the Dendroscan computer program (Varem-Sanders and Campbell, 1996), which created a density profile (Appendix Figure A.1) on which, annual growth ring measurements could be made.



Appendix Figure A.1. Sample density profile on which annual growth ring measurements were made.

The values in Appendix Table A.1 are the means and ranges of the annual growth ring measurements, and were calculated as follows. The "site mean" rows are the means of all of the measurements on each growth ring variable over all cores and all years, for each site. The "family range" rows show the minimum and maximum values of the eight family means on each site, for each growth ring variable. Finally, the "ring range" rows show the minimum and maximum values of the eleven means for each of the eleven rings measured on each core on each site, for all growth ring variables.

Appendix Table A.1. Site means and ranges for eight annual growth ring variables on each of six progeny test sites in the study.

Growth Ring Variable[a]		Series 5			Series 6		
		52	54	55	56	59	62
MXD	Site Mean	1.164	1.101	1.133	1.076	1.171	1.066
	Family Range	1.042 - 1.208	0.970 - 1.169	1.081 - 1.181	1.020 - 1.142	1.098 - 1.248	1.002 - 1.140
	Ring Range	1.022 - 1.309	0.980 - 1.190	1.051 - 1.199	0.932 - 1.138	1.045 - 1.290	0.975 - 1.143
LWD	Site Mean	0.858	0.826	0.859	0.811	0.912	0.784
	Family Range	0.716 - 0.907	0.775 - 0.950	0.787 - 0.912	0.746 - 0.876	0.860 - 0.965	0.736 - 0.869
	Ring Range	0.784 - 0.957	0.755 - 0.911	0.804 - 0.916	0.731 - 0.859	0.847 - 1.071	0.719 - 0.859
LWP	Site Mean	0.258	0.372	0.278	0.307	0.351	0.353
	Family Range	0.216 - 0.335	0.328 - 0.425	0.241 - 0.303	0.254 - 0.359	0.327 - 0.414	0.295 - 0.448
	Ring Range	0.213 - 0.319	0.285 - 0.456	0.185 - 0.366	0.230 - 0.456	0.248 - 0.463	0.251 - 0.439
LWW	Site Mean	0.195	0.264	0.176	0.206	0.158	0.257
	Family Range	0.148 - 0.278	0.178 - 0.311	0.147 - 0.207	0.145 - 0.277	0.122 - 0.196	0.210 - 0.359
	Ring Range	0.072 - 0.276	0.220 - 0.350	0.074 - 0.221	0.036 - 0.278	0.049 - 0.237	0.085 - 0.319
EWW	Site Mean	0.569	0.455	0.464	0.493	0.301	0.497
	Family Range	0.316 - 0.643	0.357 - 0.534	0.372 - 0.573	0.397 - 0.587	0.254 - 0.349	0.427 - 0.576
	Ring Range	0.255 - 0.783	0.293 - 0.597	0.152 - 0.595	0.142 - 0.674	0.059 - 0.410	0.143 - 0.693
EWD	Site Mean	0.382	0.387	0.397	0.444	0.483	0.402
	Family Range	0.316 - 0.448	0.344 - 0.415	0.312 - 0.452	0.412 - 0.492	0.451 - 0.512	0.360 - 0.495
	Ring Range	0.342 - 0.498	0.355 - 0.475	0.324 - 0.582	0.381 - 0.622	0.397 - 0.640	0.347 - 0.573
LWM	Site Mean	4.728	5.877	3.407	3.598	2.372	5.262
	Family Range	3.033 - 5.984	3.936 - 8.510	2.607 - 4.409	1.982 - 4.741	1.434 - 2.984	3.850 - 7.582
	Ring Range	0.131 - 9.675	0.515 - 10.469	0.088 - 7.753	0.048 - 8.022	0.027 - 4.990	0.091 - 10.163
TRM	Site Mean	9.552	9.724	6.483	7.449	8.021	9.303
	Family Range	4.639 - 11.414	5.490 - 13.444	5.462 - 7.784	4.622 - 9.945	6.425 - 9.475	7.176 - 11.658
	Ring Range	0.259 - 18.336	0.612 - 16.820	0.127 - 12.400	0.098 - 15.136	1.370 - 19.013	0.125 - 16.415

[a] MXD = maximum density
LWD = latewood density
LWP = latewood proportion
LWW = latewood width
EWW = earlywood width
EWD = earlywood density
LWM = latewood mass
TRM = total ring mass

APPENDIX B: Climate Data

Annual SMD values used in the regression analyses were calculated by Dr. David Spittlehouse of the British Columbia Ministry of Forests, using a water balance model developed for coastal British Columbia (Spittlehouse and Black, 1981; Spittlehouse, 1985; Giles et al., 1985). The water balance model requires inputs of climate and site data to calculate monthly SMD. All of the climate data required were obtained by Dr. Spittlehouse from Environment Canada, and included mean minimum and maximum monthly air temperatures, total solar radiation, and total rainfall, interpolated from data obtained from nearby weather stations with appropriate adjustments based on elevation, aspect, and local knowledge. An estimate of leaf area index (LAI) based on a typical value for young Douglas-fir stands was also provided by Dr. Spittlehouse. The required site data were obtained in the field by myself and Dr. Thimmappa Anekonda. These data include slope, aspect, latitude, root zone depth, and percent stone content. A single soil sample was taken on each site in the area where the trees were sampled. A location was chosen that reflected the slope and aspect of the site, as adequately as a single location can. A soil pit was in order to establish the depth of the root zone. Soil samples were taken from each horizon layer, and the soil type determined by hand in the laboratory.

The total annual SMD values for all eight sites sampled, as well as the yearly inputs for the water balance model are presented in Appendix Tables B.1 and B.2a - B.2h below.

Appendix Table B.1. Calculated estimates of yearly growing season (April-October) soil moisture deficits (1985-1995) for the eight progeny test sites included in the study.

	Site 45	Site 52	Site 54	Site 55	Site 56	Site 58	Site 59	Site 62
1985	14.3	227.3	77.6	132.2	161.4	0	215.6	162.6
1986	19.1	196.5	84.4	37.2	79.2	0	92.6	79.9
1987	22.2	250.1	87.0	57.8	22.7	0	120.3	22.6
1988	0	174.9	61.0	42.9	20.5	0	85.3	21.2
1989	0	180.7	53.8	105.9	27.9	0	54.1	29.6
1990	0	144.1	38.6	55.8	66.5	0	90.7	66.0
1991	0	155.7	22.4	21.4	42.1	0	39.0	44.9
1992	0	181.3	23.0	78.9	104.0	0	133.4	106.5
1993	0	118.9	0	0	9.6	0	32.8	10.1
1994	0	212.4	68.2	58.2	68.6	0	70.9	70.5
1995	0	165.7	35.8	75.9	80.6	0	41.7	83.5
Mean	5.05	182.5	50.2	60.6	62.1	0.0	88.8	63.4

Appendix Table B.2 a - h. Estimated growing season (April - October) climate data for the eight progeny test sites sampled in British Columbia, where:

INCPT = total intercepted rainfall by canopy in mm[a]
INFLT = total amount of water reaching the soil (infiltration) in mm
EMAX = total calculated potential energy limited evapotranspiration in mm[b]
ET = total calculated soil moisture limited (actual) evapotranspiration in mm
DEF = difference of EMAX - ET (annual total soil moisture deficit) in mm
DRN = total drainage from the soil profile in mm
RNET = average daily net solar radiation in mega joules/meter^2/day
TEMP = grand average of monthly averages of daily temperature in °C
RAIN = total growing season (April-October) rainfall in mm

[a] Dependent on LAI. The same LAI value was used for all sites, based on a value for young Douglas-fir forests.
[b] Calculated from the Priestly-Taylor equation with $\alpha = 0.8$

Appendix Table B.2a. Estimated yearly climate data for site 45

DATE	INCPT	INFLT	EMAX	ET	DEF	DRN	RNET	TEMP	RAIN
1985	160.3	59.7	279.9	265.5	14.3	794.1	59.2	8.5	1219.9
1986	116.9	660.0	251.4	232.2	19.1	427.8	52.3	9.4	776.9
1987	94.4	522.5	274.9	252.8	22.2	317.9	56.8	9.1	616.9
1988	185.2	173.0	254.3	254.3	0.0	918.7	55.4	9.6	1358.2
1989	121.9	688.4	261.2	261.2	0.0	427.2	55.1	9.1	810.2
1990	185.6	193.0	251.6	251.6	0.0	941.4	53.8	9.7	1378.7
1991	163.6	51.5	241.3	241.3	0.0	810.2	52.5	9.3	1215.0
1992	165.7	36.0	267.7	267.7	0.0	768.3	56.9	9.4	1201.7
1993	173.7	82.3	213.0	213.0	0.0	869.3	51.8	7.6	1255.9
1994	171.0	70.9	268.8	268.8	0.0	802.1	56.0	10.1	1242.0
1995	155.3	954.9	250.5	250.5	0.0	704.3	57.7	7.6	1110.1

Appendix Table B.2b. Estimated yearly climate data for site 52

DATE	INCPT	INFLT	EMAX	ET	DEF	DRN	RNET	TEMP	RAIN
1985	58.8	286.9	385.7	158.4	227.3	128.5	60.9	13.84	345.7
1986	45.4	194.4	352.9	156.4	196.5	38	53.9	15.02	239.8
1987	36	146	396.9	146.8	250.1	26.9	59.7	14.58	182.1
1988	68.8	322.4	362.2	187.3	174.9	135.1	55.8	14.6	391.3
1989	47.6	201.6	369.5	188.8	180.7	12.7	55.7	14.3	249.2
1990	69.9	334.5	358.8	214.6	144.1	119.9	54.4	15.08	404.5
1991	63	299.7	359.6	203.9	155.7	101.5	52.8	14.98	362.7
1992	63.1	292.7	377	195.7	181.3	96.9	57.9	14.64	355.7
1993	70	333.9	320.5	201.7	118.9	132.2	53.9	15.225	403.8
1994	63.2	291.8	381.5	169.1	212.4	122.7	57.8	15.6	355.1
1995	60.2	277.5	357.6	191.9	165.7	85.6	58	15.625	337.7

Appendix Table B.2c. Estimated yearly climate data for site 54

DATE	INCPT	INFLT	EMAX	ET	DEF	DRN	RNET	TEMP	RAIN
1985	98.8	568.3	282.5	204.9	77.6	363.4	46.3	14.3	667.1
1986	73.5	363.4	259.2	174.8	84.4	188.6	41.8	15.6	437.0
1987	59.1	282.3	275.7	188.7	87.0	132.3	44.4	15.1	341.4
1988	113.4	620.9	264.0	202.9	61.0	418.0	43.2	15.1	734.3
1989	77.2	379.9	263.6	209.8	53.8	170.1	42.1	14.8	457.1
1990	115.1	643.4	260.4	221.8	38.6	421.6	41.8	15.6	758.4
1991	103.0	572.8	249.6	227.3	22.4	345.6	40.4	15.5	675.8
1992	103.5	561.9	273.0	250.0	23.0	312.0	44.1	15.2	665.4
1993	111.9	614.3	219.5	219.5	0.0	394.8	40.7	15.8	726.2
1994	104.4	565.1	277.1	208.9	68.2	356.2	44.2	16.1	669.5
1995	98.1	527.1	251.2	215.4	35.8	311.7	44.2	16.2	625.1

Appendix Table B.2d. Estimated yearly climate data for site 55

DATE	INCPT	INFLT	EMAX	ET	DEF	DRN	RNET	TEMP	RAIN
1985	74.1	380.2	372.5	240.3	132.2	139.9	12.6	13.4	454.3
1986	75.0	359.7	357.2	320.0	37.2	84.0	11.6	13.1	434.7
1987	74.6	352.5	401.6	343.8	57.8	72.4	12.7	13.6	427.1
1988	85.3	411.9	364.4	321.5	42.9	90.3	11.8	12.7	497.2
1989	67.0	317.8	394.3	288.4	105.9	29.4	12.4	13.6	384.8
1990	78.8	394.8	369.6	313.8	55.8	81.0	11.7	14.3	473.6
1991	79.1	404.6	364.5	343.0	21.4	102.0	11.4	13.7	483.7
1992	97.7	511.3	383.4	304.5	78.9	206.8	12.1	14.7	609.0
1993	81.3	389.7	351.0	351.0	0.0	57.5	11.3	14.1	471.0
1994	72.5	338.6	382.8	324.7	58.2	13.9	12.0	14.5	411.1
1995	78.8	385.9	376.1	300.2	75.9	85.6	11.9	14.6	464.7

Appendix Table B.2e. Estimated yearly climate data for site 56

DATE	INCPT	INFLT	EMAX	ET	DEF	DRN	RNET	TEMP	RAIN
1985	97.6	545.6	370.6	209.2	161.4	336.4	60.4	14.2	643.1
1986	93.3	482.0	340.3	261.0	79.2	220.9	54.5	13.8	575.3
1987	99.2	505.2	339.2	316.5	22.7	188.7	53.8	14.6	604.4
1988	116.0	614.1	303.9	283.4	20.5	330.7	49.5	13.3	730.0
1989	108.5	573.8	379.5	351.6	27.9	222.2	58.9	14.4	682.3
1990	116.2	672.7	354.5	288.0	66.5	384.7	55.5	15.1	788.9
1991	93.0	483.0	352.8	310.7	42.1	172.3	55.9	14.0	576.0
1992	123.0	700.7	357.3	253.3	104.0	447.4	57.1	15.0	823.7
1993	116.9	637.4	315.1	305.5	9.6	331.9	51.2	14.3	754.4
1994	114.2	626.2	312.4	243.8	68.6	382.4	50.0	14.8	740.4
1995	124.9	701.8	344.2	263.6	80.6	438.2	54.9	15.1	826.7

Appendix Table B.2f. Estimated yearly climate data for site 58

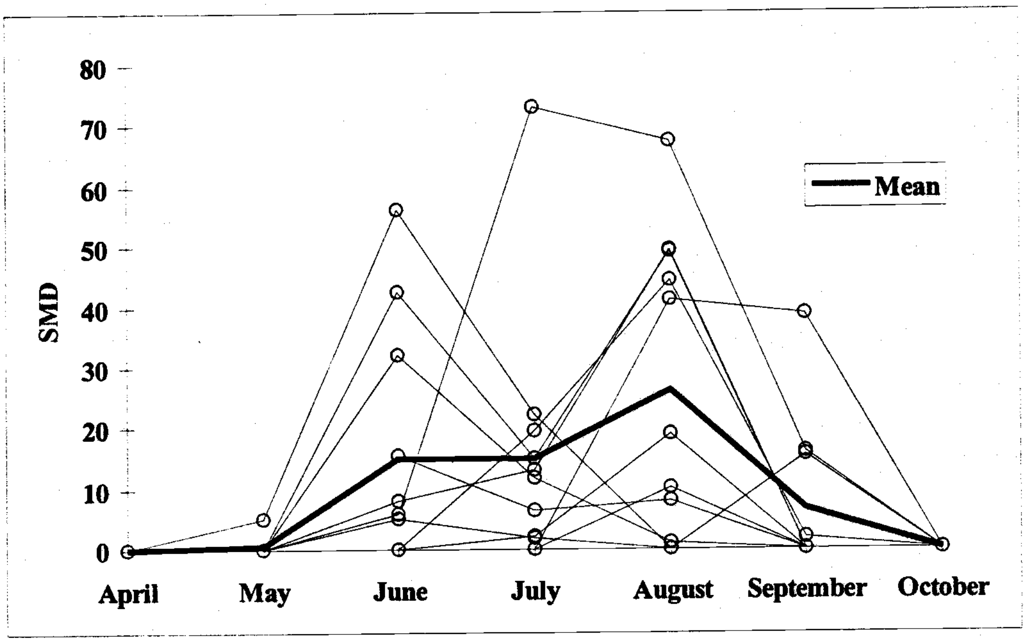
DATE	INCPT	INFLT	EMAX	ET	DEF	DRN	RNET	TEMP	RAIN
1985	152.5	941.5	327.0	327.0	0.0	614.5	62.3	12.8	1094.1
1986	148.4	862.5	296.3	296.3	0.0	566.1	58.0	12.9	1010.9
1987	100.2	530.4	342.5	342.5	0.0	243.3	63.5	13.6	630.6
1988	188.8	154.7	300.5	300.5	0.0	854.1	59.7	13.2	1343.4
1989	143.0	819.6	318.8	318.8	0.0	500.7	60.9	13.5	962.6
1990	144.6	845.2	310.5	310.5	0.0	534.6	58.0	13.8	989.9
1991	141.0	815.6	299.8	299.8	0.0	515.7	58.2	13.0	956.6
1992	148.3	847.4	331.5	331.5	0.0	515.9	62.5	13.9	995.6
1993	149.8	877.0	294.2	294.2	0.0	582.8	56.9	13.7	1026.8
1994	148.9	874.5	330.7	330.7	0.0	543.9	62.4	13.9	1023.3
1995	147.4	854.0	330.4	330.4	0.0	523.6	62.1	13.9	1001.4

Appendix Table B.2g. Estimated yearly climate data for site 59

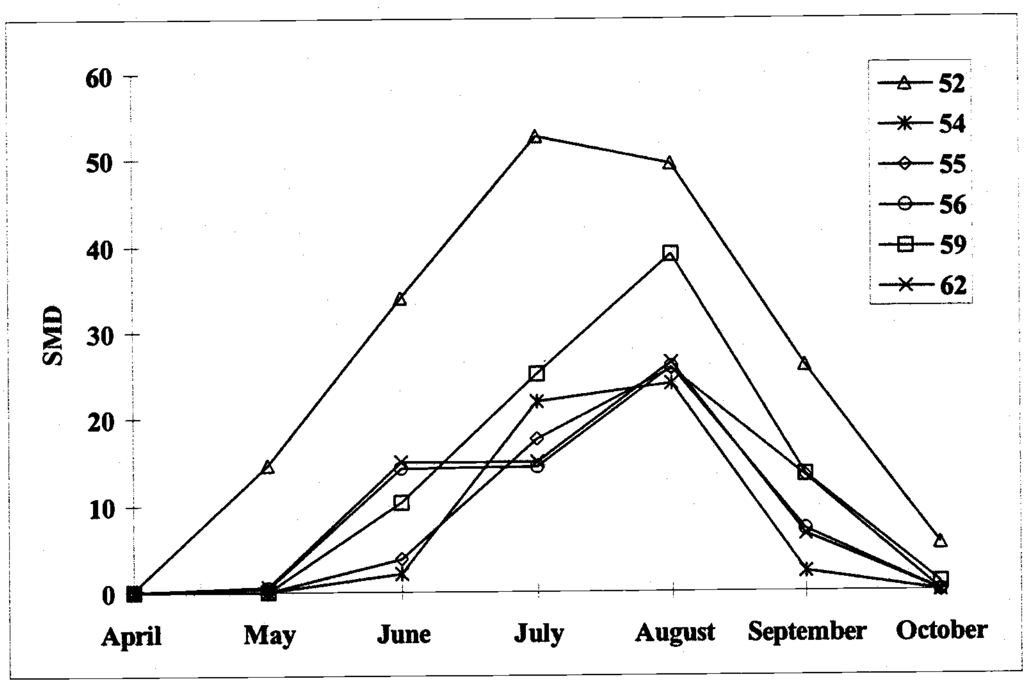
DATE	INCPT	INFLT	EMAX	ET	DEF	DRN	RNET	TEMP	RAIN
1985	65.8	318.1	424.7	209.1	215.6	109.0	67.4	13.9	383.9
1986	76.9	364.3	397.1	304.5	92.6	75.0	63.8	13.7	441.2
1987	57.4	253.8	409.1	288.7	120.3	32.3	68.5	14.1	311.2
1988	86.0	416.0	383.5	298.2	85.3	117.9	61.5	13.5	502.0
1989	77.7	365.1	395.0	340.9	54.1	24.2	61.5	13.8	442.8
1990	85.6	438.5	386.3	295.6	90.7	143.0	60.8	14.7	524.1
1991	73.1	351.9	380.5	341.5	39.0	49.0	58.3	13.9	425.0
1992	93.8	480.8	401.5	268.2	133.4	212.6	63.9	14.3	574.6
1993	92.4	478.1	362.8	329.9	32.8	177.6	59.1	14.0	570.5
1994	87.5	427.5	392.7	321.8	70.9	105.7	62.1	14.4	515.0
1995	88.5	440.5	408.1	366.4	41.7	74.1	64.0	14.6	529.0

Appendix Table B.2h. Estimated yearly climate data for site 62

DATE	INCPT	INFLT	EMAX	ET	DEF	DRN	RNET	TEMP	RAIN
1985	97.6	545.6	372.8	210.2	162.6	335.3	60.1	14.7	643.1
1986	93.3	482.0	341.4	261.6	79.9	220.4	54.2	14.3	575.3
1987	99.2	505.2	341.1	318.5	22.6	186.8	53.6	15.1	604.4
1988	116.0	614.1	305.5	284.3	21.2	329.8	49.4	13.8	730.0
1989	108.5	573.8	381.1	351.5	29.6	222.3	58.6	14.9	682.3
1990	116.2	672.7	356.7	290.7	66.0	382.0	55.3	15.6	788.9
1991	93.0	483.0	354.0	309.0	44.9	174.0	55.8	14.5	576.0
1992	123.0	700.7	360.1	253.6	106.5	447.1	57.0	15.5	823.7
1993	116.9	637.4	316.0	305.9	10.1	331.5	51.0	14.8	754.4
1994	114.2	626.2	314.4	243.8	70.5	382.4	49.9	15.3	740.4
1995	124.9	701.8	347.4	263.9	83.5	437.9	54.7	15.6	826.7



Appendix Figure B.1. Estimated monthly SMD values throughout growing season on site 62 over an 11 years (1985-1995) period.

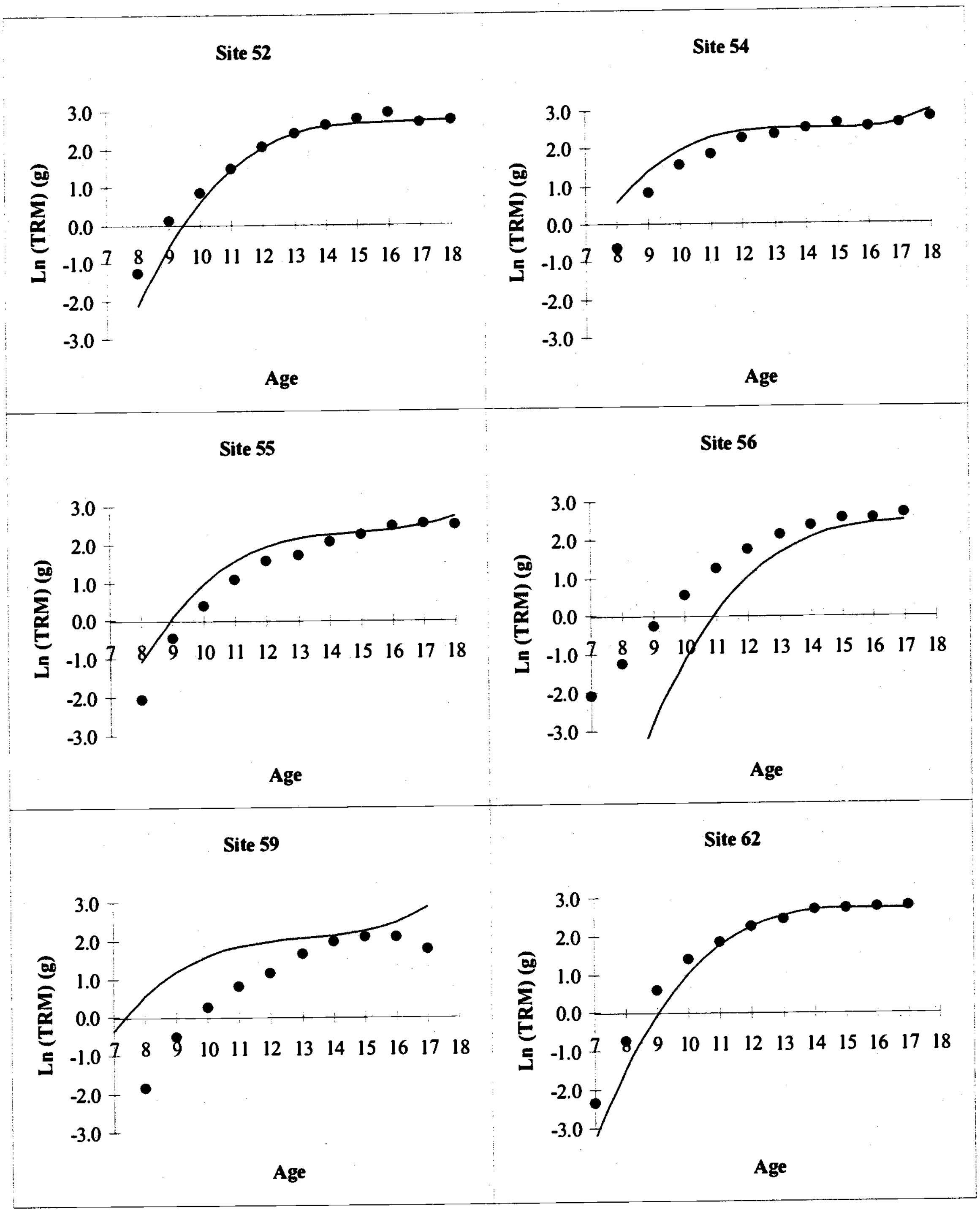


Appendix Figure B.2. Mean estimated monthly SMD values throughout the growing season on each of six sites in the study.

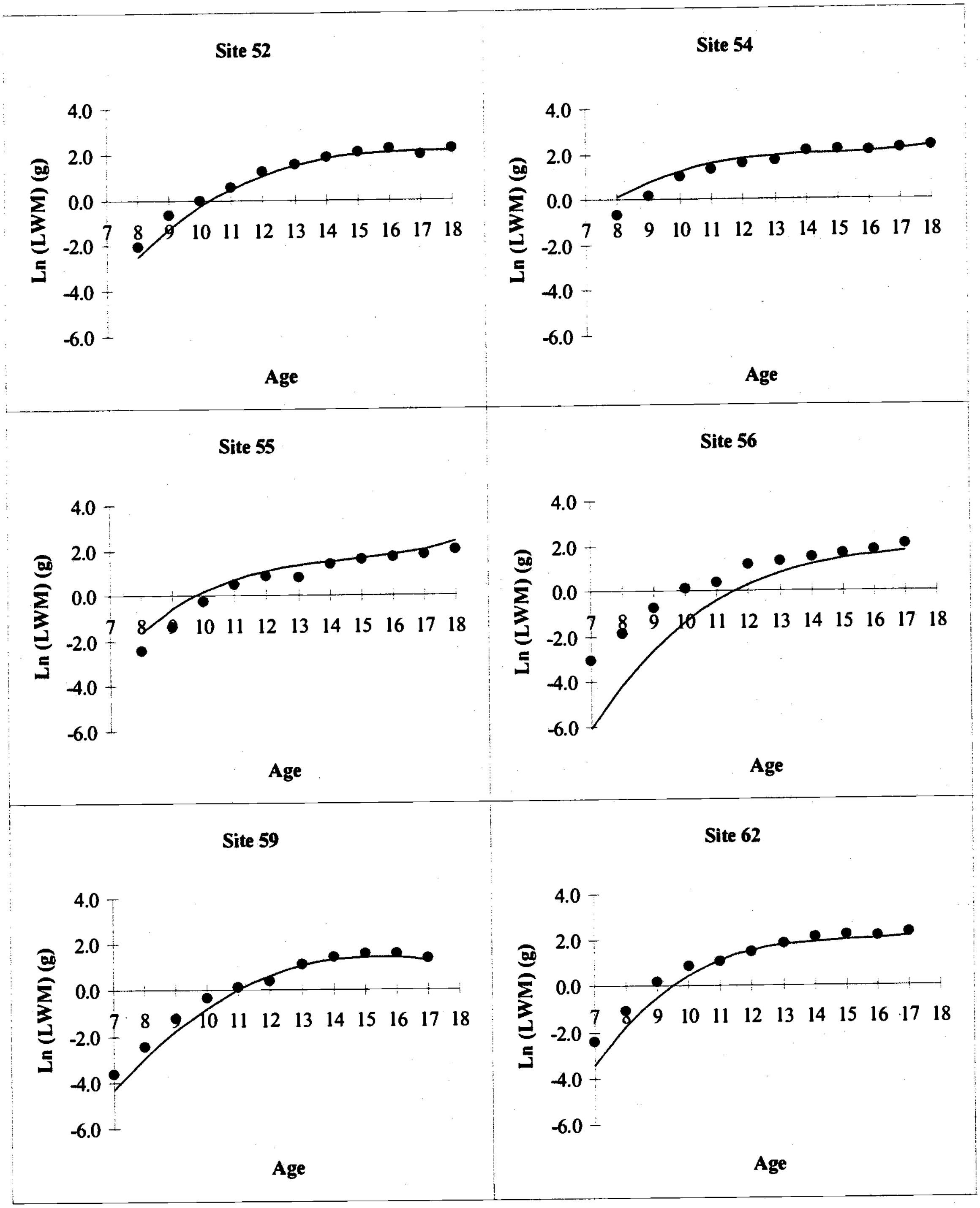
APPENDIX C: Age Trends

Age Trends for the Five Growth Ring Variables [MXD, ln(TRM), ln(LWW), ln(LWM), ln(EWW)] Not Presented in Chapter 2.

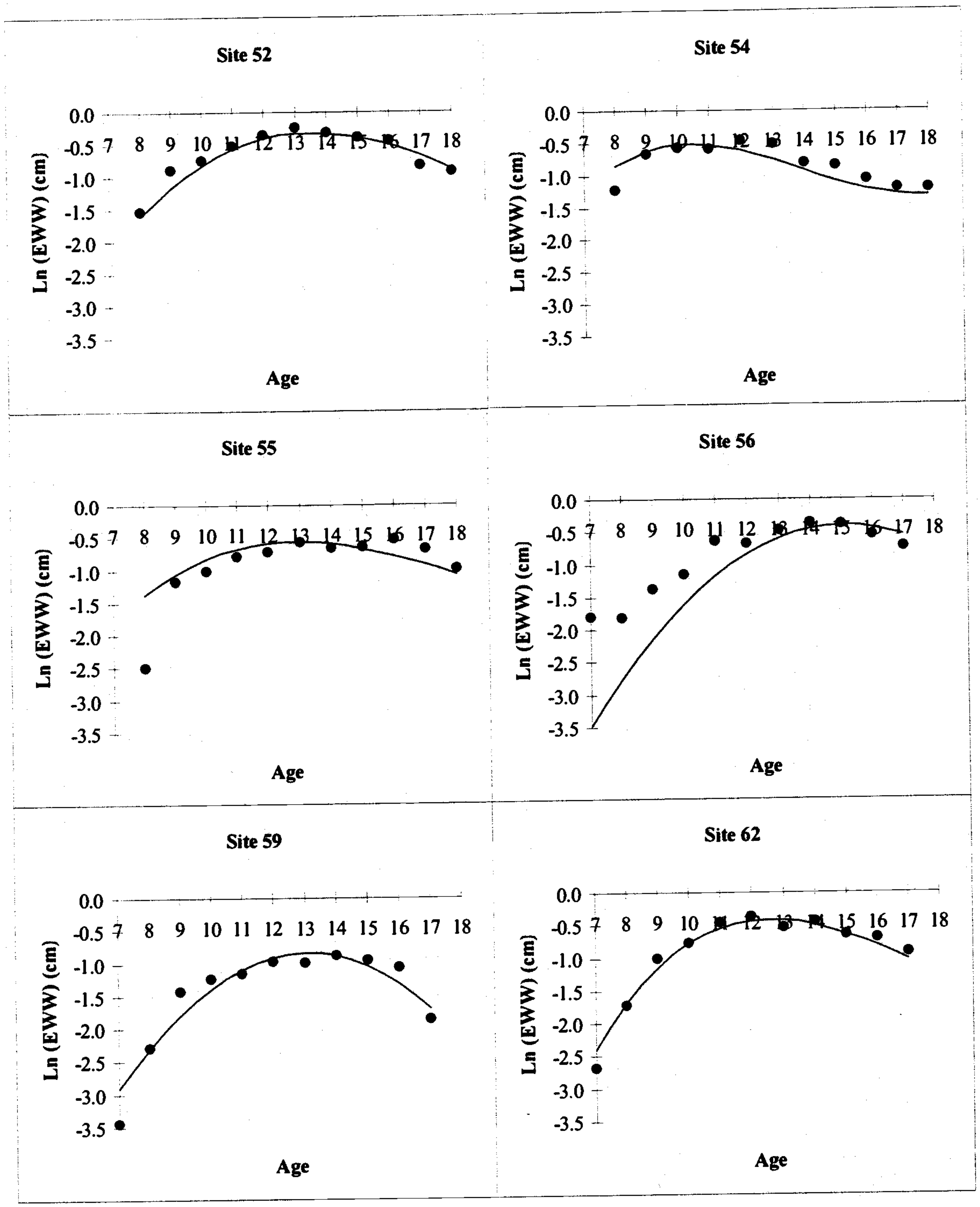
The curves in Appendix Figures C.1a - e, in addition to Figures 2.6a - 2.6c in chapter 2, show the age-trends determined from the regression analysis of each of the annual growth ring variables for each site. The curves are derived from the estimated parameters in the full "age model" (where the ring variable was regressed on source, age, and relationship variables, without SMD, using data from all sites in the same analysis).



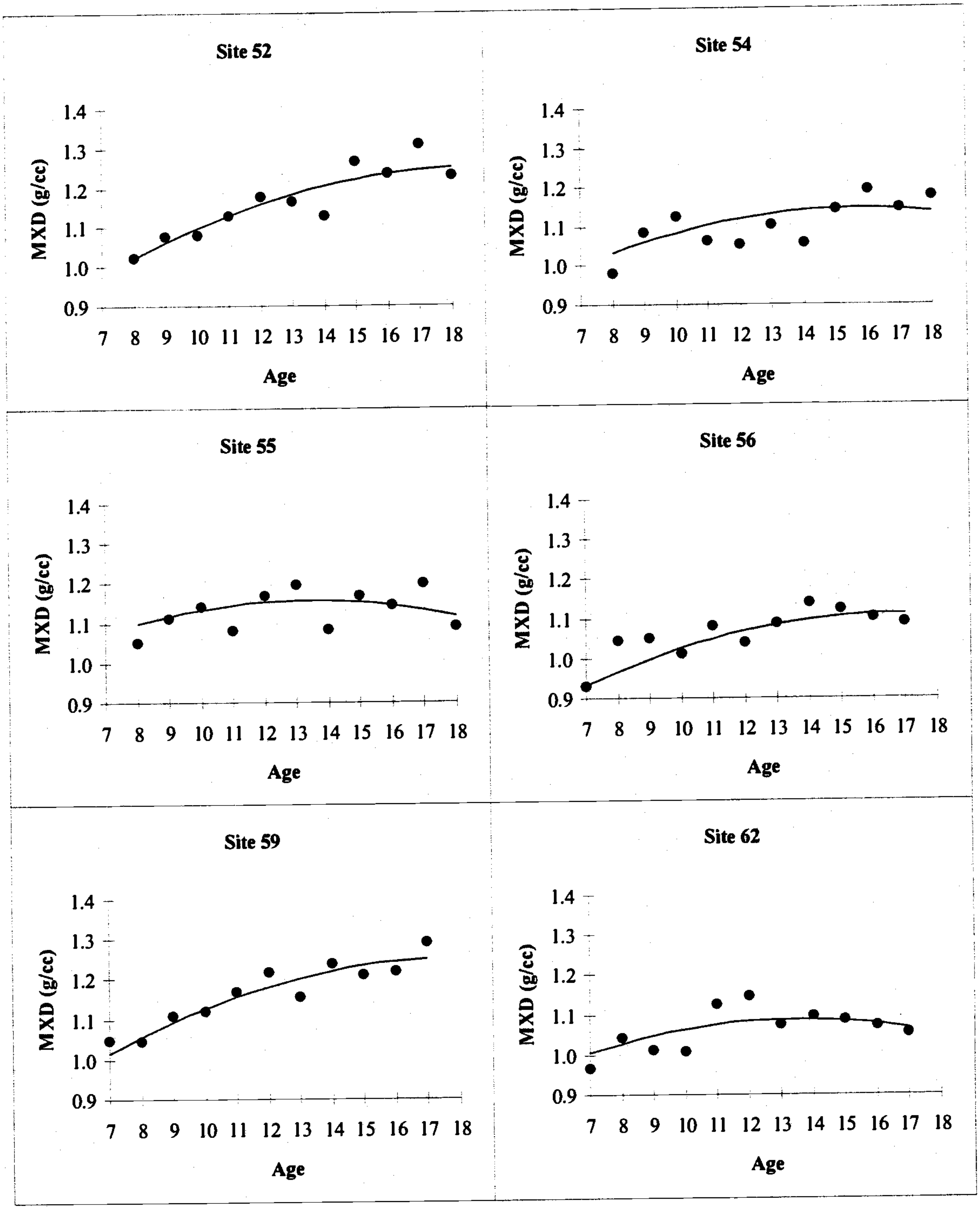
Appendix Figure C.1a. Age-trends by site for ln[Total Ring Mass (TRM)].



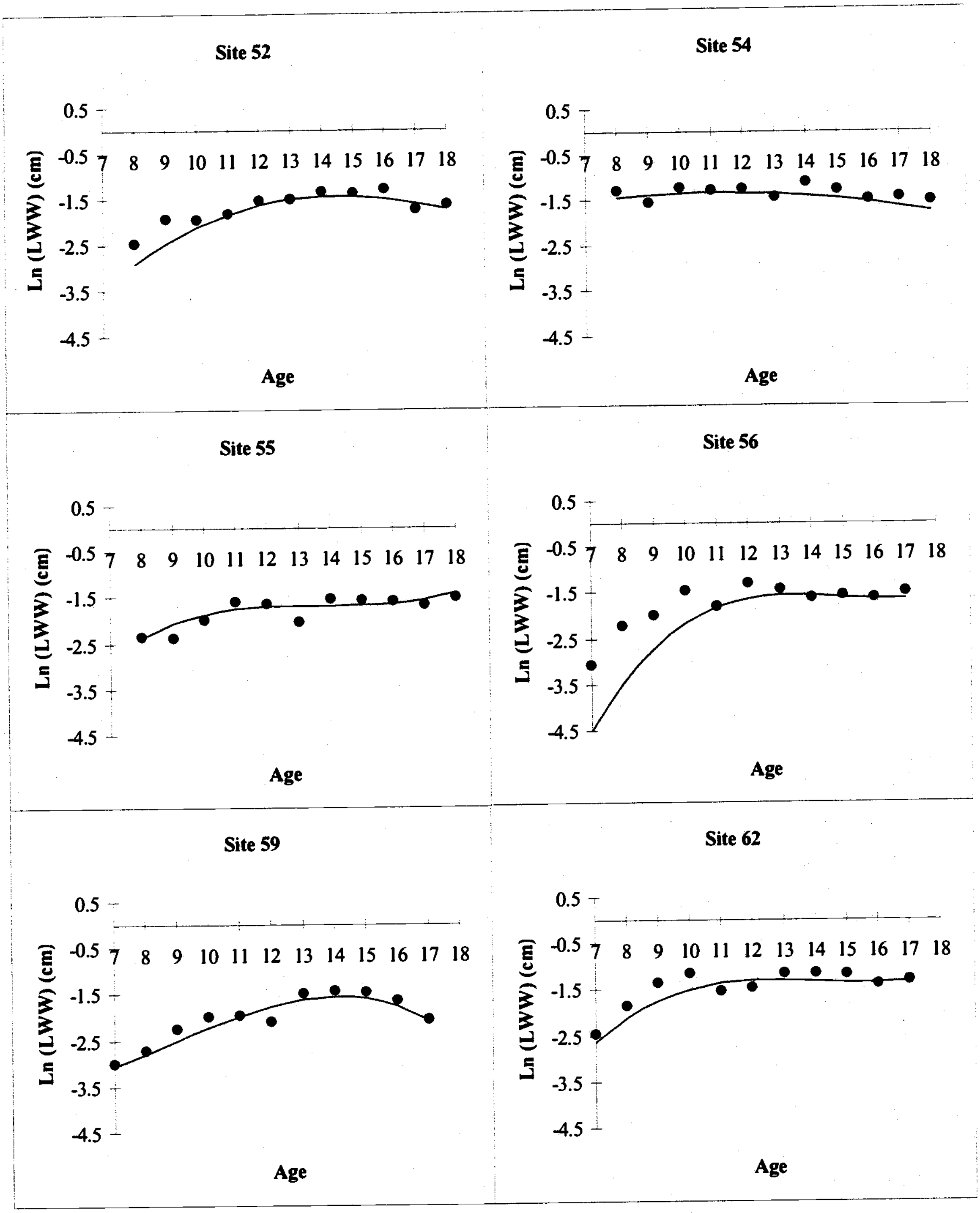
Appendix Figure C.1b. Age-trends by site for ln[Latewood Mass (LWM)].



Appendix Figure C.1c. Age-trends by site for ln[Earlywood Width (EWW)].



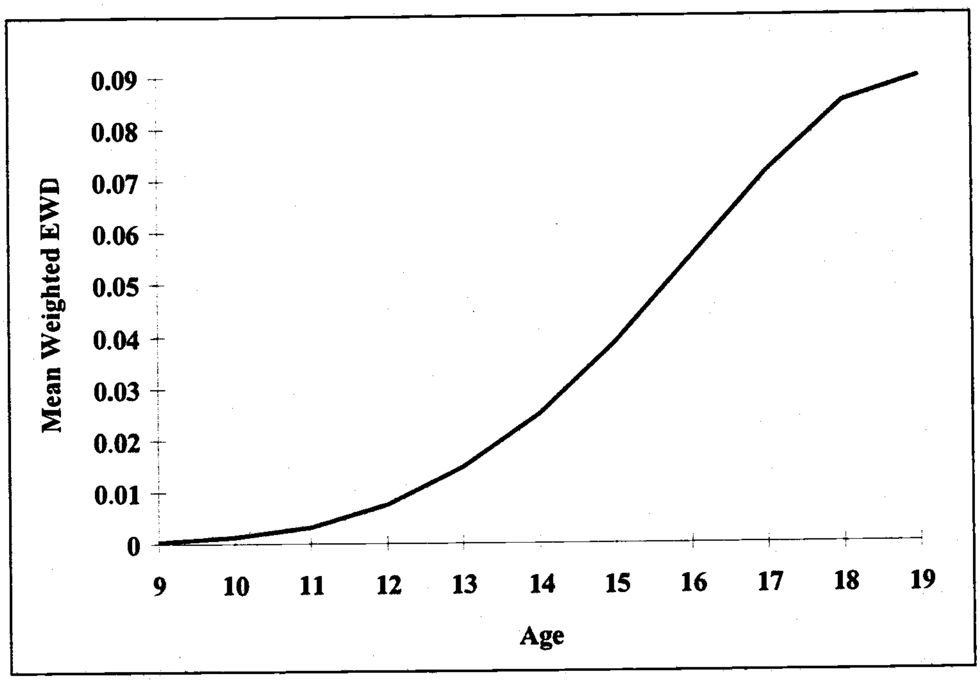
Appendix Figure C.1d. Age-trends by site for Maximum Density [MXD].



Appendix Figure C.1e. Age-trends by site fore ln[Latewood Width (LWW)].

Earlywood Weighted by Method of Vargas-Hernandez et al. (1994)

Appendix Figure C.2 shows the mean age trend for earlywood density from site 52, when weighted by dividing the surface area of the earlywood in each ring by the total surface area of the tree (Vargas-Hernandez et al., 1994).

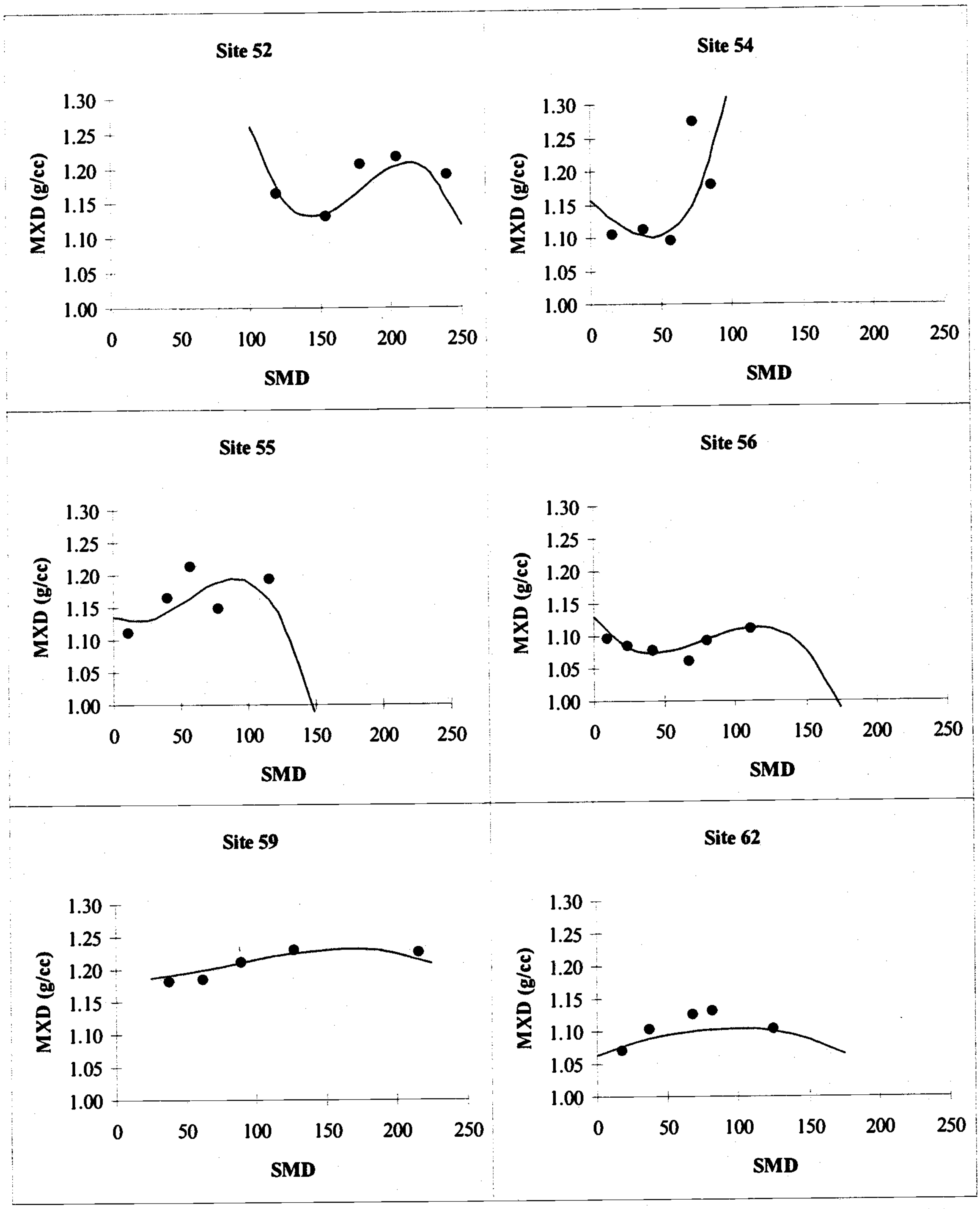


Appendix Figure C.2. Mean weighted earlywood density (EWD) by ring age for site 52.

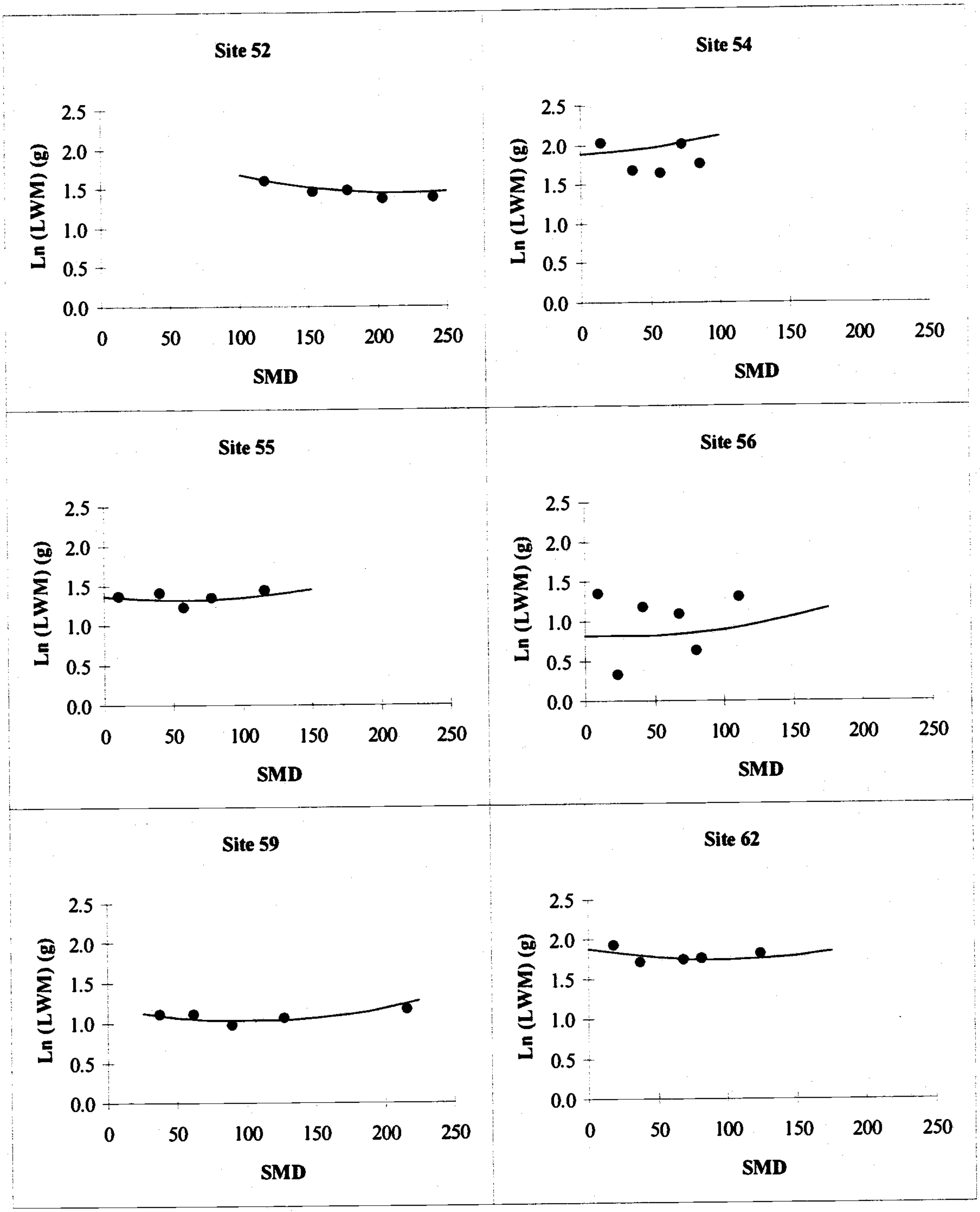
APPENDIX D: Regressions Curves of Ring Variables on SMD

Regressions of four ring variables on SMD with inconsistent associations across the six test sites

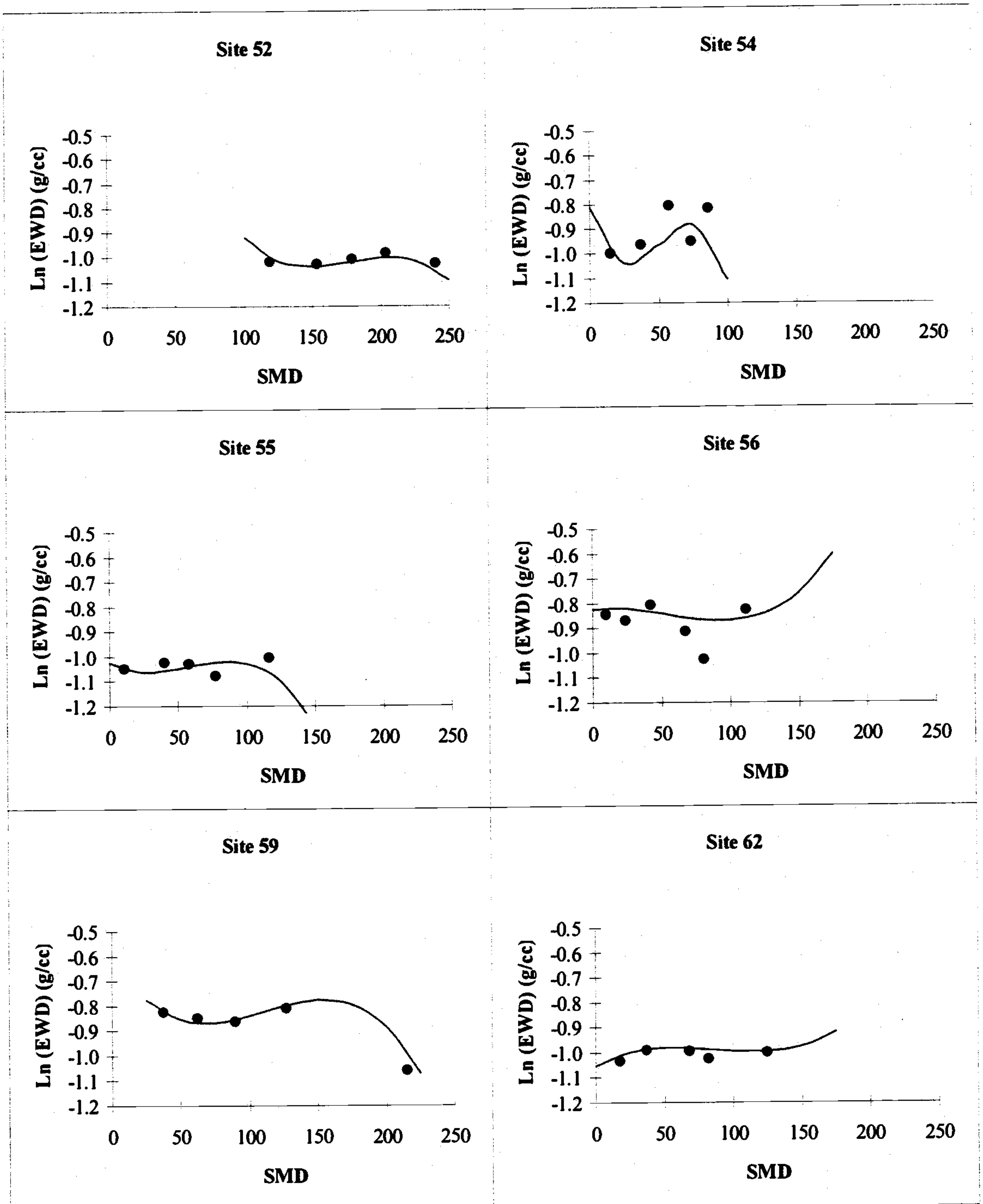
The regression curves in Appendix Figures D.1a - d, in addition to Figures 2.6 - 2.9 in chapter 2, clearly show the differences across sites in shape as a result of age x SMD interactions. In most cases, a general effect of SMD on each response variable is evident from the curves, however, the lack of consistency in the responses over sites is also visible. The curves were plotted from the calculated values for each response variable at a given age (13) over the range of SMD experienced on each site. The data points were calculated in a similar fashion, using an indicator variable for five or six SMD classes determined individually on each site.



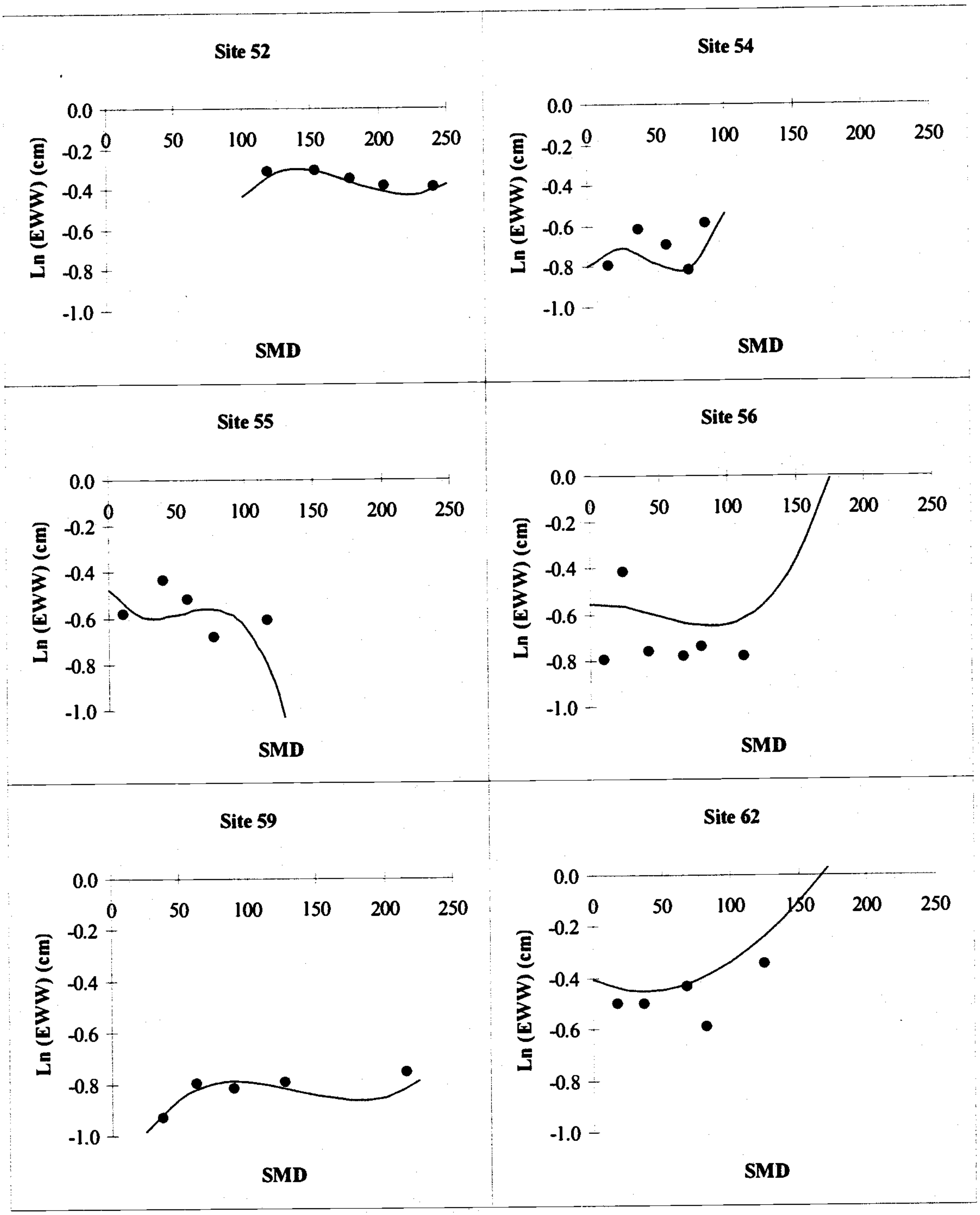
Appendix Figure D.1a. Regressions of Maximum Density (MXD) on Soil Moisture Deficit (SMD) for each of six test sites.



Appendix Figure D.1b. Regressions of ln[Latewood Mass (LWM)] on Soil Moisture Deficit (SMD) for each of six test sites.



Appendix Figure D.1c. Regressions of ln[Earlywood Density (EWD)] on Soil Moisture Deficit (SMD) for each of six test sites.



Appendix Figure D.1d. Regressions of ln[Earlywood Width (EWW)] on Soil Moisture Deficit (SMD) for each of six test sites.

APPENDIX E: Growth Ring Variable Correlations

The correlations in Appendix Table E.1 were calculated over all cores for each of the eleven years (1985-1995) in the analysis, and then averaged over the six sites.

Appendix Table E.1. Pearson correlation coefficients between growth ring variables.

	CORRELATION COEFFICIENTS						
	LWD	LWP	ln(LWW)	ln(LWM)	ln(TRM)	ln(EWW)	ln(EWD)
LWP[a]	-0.34						
ln(LWW)	-0.31	0.54					
ln(LWM)	0.12	0.05	0.65				
ln(TRM)	0.22	-0.03	0.60	0.93			
ln(EWW)	0.06	-0.52	0.42	0.59	0.64		
ln(EWD)	0.28	-0.02	-0.41	-0.44	-0.37	-0.40	
MXD	0.66	-0.17	0.02	0.30	0.39	0.19	0.09

[a] See Appendix Table A.1 for abbreviations.

APPENDIX F: SAS Output And Regression Models

Below are examples of the SAS code used to perform the regression analyses on the data combined over all six sites. The first example is the code for the regression analysis with the deficit (SMD) terms, and the second example is for the regression analysis with orthogonal contrasts for the deficit terms. The terms in italics are the terms that are changed to adjust the model for each response (annual growth ring) variable. The third example is the SAS output from the code in example 1.

All of the annual growth ring data, and the climate data will be archived in the Oregon State University Forest Science data bank. In addition, the SAS code, and examples of the output will be archived and available for retrieval.

Example F.1: SAS code for regression analysis for LWD

```
proc mixed data=alldata update noitprint  noclprint covtest;
        class core family tree site block ;
        model lw_dens = block age|age|age|site(block)
            deficit|deficit|site(block) deficit*deficit*deficit /
                  solution ddfm=bw;
        random family(site) tree(family site);
        repeated /    type=ar(1)    subject = core(tree family);
run;
```

Example F.2: SAS code for regression analysis of LWD with orthogonal contrasts for deficit terms.

```
proc mixed data=alldata update noitprint  noclprint covtest;
        class core family tree site block ;
            model lw_dens = block age|age|age|site(block)
            U1 U2 U3 U1*site(block) U2*site(block)| solution ddfm=bw;
        random family(site) tree(family site);
        repeated /    type=ar(1)    subject = core(tree family);
run;
```

Example F.3: SAS output from example 1.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
BLOCK	1	61	0.12	0.7314
AGE	1	1573	0.14	0.7114
AGE*AGE	1	1573	0.11	0.7362
AGE*AGE*AGE	1	1573	0.21	0.6435
SITE(BLOCK)	4	1573	7.26	0.0001
AGE*SITE(BLOCK)	5	1573	5.69	0.0001
AGE*AGE*SITE(BLOCK)	5	1573	5.83	0.0001
AGE*AGE*AGE*SIT(BLO)	5	1573	6.13	0.0001
DEFICIT	1	1573	20.49	0.0001
DEFICIT*DEFICIT	1	1573	2.34	0.1263
DEFICIT*SITE(BLOCK)	5	1573	3.99	0.0013
DEFI*DEFI*SITE(BLOC)	5	1573	4.30	0.0007

Covariance Parameter Estimates (REML)

Cov Parm	Subject	Estimate	Std Error	Z	Pr > |Z|
FAMILY(SITE)		0.00076673	0.00057433	1.34	0.1819
TREE(FAMILY*SITE)		0.00217875	0.00057902	3.76	0.0002
AR(1)	CORE(FAMILY*TREE)	0.20638518	0.02867189	7.20	0.0001
Residual		0.00713811	0.00027888	25.60	0.0001

Model Fitting Information for LW_DENS

Description	Value
Observations	1670.000
Res Log Likelihood	1477.005
Akaike's Information Criterion	1473.005
Schwarz's Bayesian Criterion	1462.208
-2 Res Log Likelihood	-2954.01

Solution for Fixed Effects

Effect	SITE	BLOCK	Estimate	Std Error	DF	t	Pr > |t|
INTERCEPT			0.84303995	0.56294685	61	1.50	0.1394
BLOCK		1	0.40804145	0.71091799	61	0.57	0.5681
BLOCK		2	0.00000000	.	.	.	.
AGE			-0.04748898	0.14326140	1573	-0.33	0.7403
AGE*AGE			0.00547364	0.01172683	1573	0.47	0.6407
AGE*AGE*AGE			-0.00018395	0.00031074	1573	-0.59	0.5540
SITE(BLOCK)	52	1	-1.68716547	0.61997912	1573	-2.72	0.0066
SITE(BLOCK)	54	1	-0.40922638	0.65374696	1573	-0.63	0.5314
SITE(BLOCK)	55	1	0.00000000	.	.	.	.
SITE(BLOCK)	56	2	1.84527606	0.95278253	1573	1.94	0.0530
SITE(BLOCK)	59	2	-2.25944788	0.77235757	1573	-2.93	0.0035
SITE(BLOCK)	62	2	0.00000000	.	.	.	.
AGE*SITE(BLOCK)	52	1	0.26139045	0.18048686	1573	1.45	0.1477
AGE*SITE(BLOCK)	54	1	0.01378778	0.19588616	1573	0.07	0.9439
AGE*SITE(BLOCK)	55	1	-0.08414857	0.18394769	1573	-0.46	0.6474
AGE*SITE(BLOCK)	56	2	-0.40653590	0.22923240	1573	-1.77	0.0763
AGE*SITE(BLOCK)	59	2	0.62433417	0.19767067	1573	3.16	0.0016
AGE*SITE(BLOCK)	62	2	0.00000000	.	.	.	.
AGE*AGE*SITE(BLOCK)	52	1	-0.02155371	0.01465235	1573	-1.47	0.1415
AGE*AGE*SITE(BLOCK)	54	1	-0.00220857	0.01658875	1573	-0.13	0.8941
AGE*AGE*SITE(BLOCK)	55	1	0.00635763	0.01524956	1573	0.42	0.6768
AGE*AGE*SITE(BLOCK)	56	2	0.02897444	0.01787798	1573	1.62	0.1053
AGE*AGE*SITE(BLOCK)	59	2	-0.05357806	0.01623180	1573	-3.30	0.0010
AGE*AGE*SITE(BLOCK)	62	2	0.00000000	.	.	.	.
AGE*AGE*AGE*SIT(BLO)	52	1	0.00059860	0.00038508	1573	1.55	0.1203
AGE*AGE*AGE*SIT(BLO)	54	1	0.00012358	0.00045274	1573	0.27	0.7849
AGE*AGE*AGE*SIT(BLO)	55	1	-0.00014796	0.00040879	1573	-0.36	0.7174
AGE*AGE*AGE*SIT(BLO)	56	2	-0.00065558	0.00045415	1573	-1.44	0.1491
AGE*AGE*AGE*SIT(BLO)	59	2	0.00151745	0.00043162	1573	3.52	0.0005
AGE*AGE*AGE*SIT(BLO)	62	2	0.00000000	.	.	.	.
DEFICIT			0.00162396	0.00042981	1573	3.78	0.0002
DEFICIT*DEFICIT			-0.00000835	.	.	.	.
DEFICIT*SITE(BLOCK)	52	1	0.00189606	0.00115210	1573	1.65	0.1000
DEFICIT*SITE(BLOCK)	54	1	-0.00238890	0.00105236	1573	-2.27	0.0233
DEFICIT*SITE(BLOCK)	55	1	0.00032286	0.00063138	1573	0.51	0.6092
DEFICIT*SITE(BLOCK)	56	2	-0.00031927	0.00067268	1573	-0.47	0.6351
DEFICIT*SITE(BLOCK)	59	2	-0.00154409	0.00059436	1573	-2.60	0.0095
DEFICIT*SITE(BLOCK)	62	2	0.00000000	.	.	.	.
DEFI*DEFI*SITE(BLOC)	52	1	-0.00000099	0.00000436	1573	-0.23	0.8201
DEFI*DEFI*SITE(BLOC)	54	1	0.00002426	0.00001114	1573	2.18	0.0296
DEFI*DEFI*SITE(BLOC)	55	1	-0.00000301	0.00000494	1573	-0.61	0.5418
DEFI*DEFI*SITE(BLOC)	56	2	-0.00000025	0.00000540	1573	-0.05	0.9627
DEFI*DEFI*SITE(BLOC)	59	2	0.00001022	0.00000390	1573	2.62	0.0089
DEFI*DEFI*SITE(BLOC)	62	2	0.00000000	.	.	.	.