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Application of Linear Mixed Model: The Effect of Climatic Factors on the Wood Anatomy of Two Eucalypt Clones

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Authors' contributions

This work was carried out in collaboration between both authors. Authors DGA and TTZ designed the study, wrote the protocol and author DGA wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Eucalypt trees are one of tree species used for the manufacturing of papers in South Africa. The manufacturing of paper consists of cooking the wood with chemicals until obtaining a pulp. The wood is made of different cells. The shape and structure of these cells, called wood anatomical characteristics are important for the quality of paper. In addition, the anatomical characteristics of wood are influenced by environmental factors like climatic factors, soil compositions etc…. Therefore, in this study we investigated the effect of climatic factors on wood anatomical characteristics of two *Eucalyptus* clones. In the experiment, two sets of data were recorded daily, the climatic parameters and the tree growth. After cutting the trees, the anatomical properties of the wood were measured using microscope and image analysis. The longitudinal linear mixed model with age, season, temperature, rainfall, solar radiation, relative humidity and wind speed as the fixed effects factors and tree as random effect factor was fitted to the data. Lagged effects climatic variables were identified and included in the model. To account for the physical characteristics of

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the trees we included the effect of diameter at breast height (DBH), stem radius, daily radial increment, and the suppression or dominance of the tree in the model. It was found that wood anatomical characteristics of the two clones were more affected by climatic variables when the tree was on juvenile stage as compared to mature stage.

Keywords: LMM; eucalypt tree; PC.

1. INTRODUCTION

Numerous *Eucalyptus* tree species have been introduced into South Africa 85 years ago, mainly for timber and firewood, pulpwood and also for ornamental purposes [1]. The great advantage of the *Eucalyptus* trees are that they are fast growing, require little attention and when harvested regrow from the stumps to be harvested every ten years [2].

Eucalyptus pulp is a raw material for the manufacture of bulky and/or opaque papers. Therefore, the *Eucalyptus* wood is a composition of fibre and vessel elements. Fibre and vessel characteristics in wood are important features since they strongly affect the quality and performance of the final product. These two elements have different functions. The fibres have the function of the support and vessels have the function of conduction of water and nutrients. Therefore, the elements that are important to pulping are: the number of fibre per gram of pulp, individual fibre strength, fibre collapsibility, fibre bonding ability, fibre swelling and hydration, and fibre deformation [3].

In South Africa, Sappi is one of the leading suppliers of coated fine paper and chemical cellulose. The company has 550,000 hectares of *Eucalyptus* plantations in South Africa. From the total land owned by Sappi, 66% of the land is planted with *Eucalyptus* tree, and produces about 37 million tones of timber per annum. In addition to producing a wide range of coated and uncoated paper, Sappi produces tissues wadding and fibre board with an annual capacity of approximately 350,000 tons. To control and understand pulp quality, Sappi started a trial to investigate the relationship between wood anatomic property and climatic factors [4].

2. MATERIALS AND METHODS

2.1 Study Design

The data have been collected from an experiment put in place by Sappi. In July 2001, the eucalypt fibre research trial 092 (EFR092T referred to as the “dendrometer trial”) was established in costal Zulu-land in mid-2001. The experiment site of the dendrometer trial is located at Kwambonambi (Kwazulu Natal). Fundamental to the research was the aim of linking short term variations in environmental and tree physiological conditions with differences in fibre processes [5]. For the experiment, two important Sappi hybrids (*Eucalyptus grandis* × *urophylla* (GU) and *Eucalyptus grandis* × *camuldulensis* (GC)) were established. The Sappi experiment has been designed to run over at least 8 years, in separate phases. In order to measure wood anatomical properties, the trees had to be felled after a certain period of time. Then, the measurement equipment was transferred to a new set of tree. In consequence, the data have been divided into phases, i.e. Phase I, Phase II, Phase III and Phase IV. For each phase, a sample of 9 trees per clone from the research trial was selected. Moreover, these selected trees were used to investigate the physiological and morphological variables throughout the life of the stand.

2.2 Variables of Interest

2.2.1 Response variable

The eleven wood anatomical characteristics were found to be highly correlated. The appropriate procedures for investigating the effects of the climatic factors on the eleven wood anatomical properties were multivariate procedures such as multivariate analysis of variance or multivariate regression analysis rather than univariate procedures such as individual analysis of variance or regression analysis of the characteristics. This is because the eleven wood anatomical characteristics may be highly correlated as a result of being measured on the same trees. Moreover, there was correlation between wood anatomical characteristics. For example, fibre radial diameter, fibre tangential diameter and fibre lumen diameter were correlated. Alternatively, univariate procedures may be used to investigate the effects of the climatic factors on fewer than eleven linear combinations of the eleven wood anatomical characteristics. This could be achieved using principal component (PC) analysis techniques. The principal component analysis was done for each phase and clone separately. The eleven fibre and vessel characteristic variables were reduced to four principal components. The first four PCs for both the GU and GC fibre and vessel characteristics of eucalypt clones were found to be the dominant PCs. Note that for the sake of brevity, only the dominant fibre and vessel characteristics appear on the right hand side of the equation of each PC, and hence the use of "≈" in the equations.

For GC:
VD (PC1) ≈ 0.971 VTD + 0.972 VRD + 0.977 VA
FD (PC2) ≈ 0.729 FTD + 0.972 FRD + 0.717 FLD + 0.961 FA
FW (PC3) ≈ 0.966 FWA + 0.969 FWT
VF (PC4) ≈ 0.85 VF +0.915 VP

For GU:
VD (PC1) ≈ 0.981 VTD + 0.982 VRD + 0.987 VA
FD (PC2) ≈ 0.739 FTD + 0.982 FRD + 0.727 FLD +0.971 FA
FW (PC3) ≈0.976 FWA +0.979 FWT
VF (PC4) ≈ 0.86 VF + 0.925 VP

where FTD=Fibre Tangential Diameter, FRD=Fibre Radial Diameter, FLD=Fibre Lumen Diameter, FWA=Fibre Wall Area, FWT=Fibre Wall Thickness, FA=Fibre Area, VTD=Vessel Tangential Diameter, VRD=Vessel Radial Diameter, VF=Vessel Frequency, VP=Vessel Percentage (VP), and VA=Vessel Area.

Therefore, the first principal component was labelled as vessel dimension (VD). The second principal component was dominated by fibre tangential diameter, fibre radial diameter, fibre lumen diameter and fibre area; and classified as fibre dimension (FD). The third principal component was dominated by fibre wall area and fibre wall thickness; and it was labelled as fibre wall (FW). And the fourth principal component was mainly vessel frequency and vessel percentage and was labelled as vessel frequency (VF). Therefore, the objective of this paper was to find the effect of climatic factors on the wood anatomy of two *Eucalyptus* clones.

2.2.2 Independent variables

The independent covariates comprised the climatic variables (Temperature, Rainfall (seven day cumulative), Solar radiation, Relative humidity and Wind speed), age of the tree, season, diameter at breast height (DBH), radius and increment. In addition to these variables, lagged effects for climatic variables were included.

2.3 The Statistical Model

The linear mixed model (LMM) was first developed for applications in animal genetics and breading research [6-8]. The model consists of both fixed and random effects. Fixed effects are effects which can be used only if the interest is in the effects of the levels of the factors used in the experiment. On the other hand, the effect is random if the levels in the study are randomly selected and the interest in the effect of the population of the levels of a factor or factors. Repeated measurement data refers to data generated by measuring some specified characteristic(s) of the experimental/sampling unit(s) repeatedly over time. The experimental/sampling unit is called subject. Therefore, with repeated measurements, one can capture within subject changes. To account for the within subject changes of a certain response over time, the longitudinal models can be used. The term "longitudinal data" is also used to describe repeated measurements. The main objective of a longitudinal study is to characterize the change of the responses over time and the factors that influence the change of the response [9].

In general, when repeated measures of responses taken from each of subject from certain population, we can have two types of variability. These are the within subject variability and the between subject variability. For subject i=1, 2,..., k, let $\boldsymbol{y}_i = (y_{i1}, \ldots, y_{in})'$ be an $n \times 1$ vector of responses. Then the general linear mixed model for the response $\boldsymbol{y}_i$ can be written as:

$$\boldsymbol{y}_i = \boldsymbol{X}_i\beta + \boldsymbol{Z}_i\boldsymbol{u}_i + \varepsilon_i\,,\ i = 1,2,\ldots,k \qquad (1)$$

Where

β is a $p \times 1$ vector of fixed effects;
$\boldsymbol{y}_i$ is an $n \times 1$ vector of observed responses;
$\mathbf{X}_i$ is an $n \times p$ design matrix associated with β;
$\mathbf{u}_i$ is a $q_i \times 1$ vector of independent random effects with a N ($\mathbf{0}$, $\mathbf{I}_{qi}\sigma^2{}_i$) distribution;
$\mathbf{Z}_i$ is an $n \times q_i$ design matrix associated with $\mathbf{u}_i$, where $\mathbf{u}_i$ is a $q_i \times 1$ vector of independent random variables with a N(0, σ_i^2) distribution, i = 1, 2, . . . ,k,
$\boldsymbol{\varepsilon}_i$ is an n × 1 vector of random errors from a N (0, $\boldsymbol{I}_n\sigma_e^2$), and $\mathbf{u}_i$ and $\boldsymbol{\varepsilon}_i$ are mutually independent.

The random effects vectors $\mathbf{u}_i$ are assumed to be independent and normally distributed with mean vector **0** and variance – covariance **G**, i.e. **u** = [$\mathbf{u}_1$' |$\mathbf{u}_2$'|. . . |$\mathbf{u}_k$'] ~ N(**0**, **G**), where **G** is a block diagonal with the ith block $\sigma_i^2 \boldsymbol{I}_{qi}$, and the error vectors $\boldsymbol{\varepsilon}_i$ are assumed to be independent and normally distributed with mean vector **0** and variance – covariance matrix $\mathbf{R}_i$, i.e. $\boldsymbol{\varepsilon}_i \sim N(\mathbf{0}, \boldsymbol{R}_i)$, for i = 1, 2, . . ., k. Here, **G** and $\mathbf{R}_i$ are $q \times q$ (where, $q=q_1 + q_2 + ... + q_k$) and $n \times n$ matrices respectively. Under the assumption of normality and independence for $\mathbf{u}_i$ and $\boldsymbol{\varepsilon}_i$, the marginal distribution of the response y_i is normal with mean **X**β and variance – covariance matrix V_i where $V_i = \sigma_e^2 \boldsymbol{I}_n + \boldsymbol{ZGZ}' = \sigma_e^2 \boldsymbol{I}_n + \sum_{i=1}^{r} \sigma^2 \boldsymbol{Z}_i \boldsymbol{Z}_i'$ [10,11]. Estimation of σ_e^2 -and the σ_i^2 is done using either the analysis of variance (ANOVA) method, or the maximum likelihood and the restricted/residual maximum likelihood methods under the assumption of normality and independence for $\mathbf{u}_i$ and ε_i. The methods are described in the next section. Further literature for linear mixed model can be found in different books [7,8,12-20].

3. RESULTS

We investigate the effects of the climatic factors on the wood anatomical characteristics represented by principal components (PCs): Vessel Dimension (VD), Fibre Dimension (FD), Fibre Wall (FW) and Vessel Frequency (VF). The effect of climatic conditions on the wood fibre and vessel characteristics of *Eucalyptus* tree is assessed by accounting for the effect of the age of the tree. Accordingly, we fit a mixed model with the age and climatic variables as fixed effects and trees as random effects. The climatic variable effects include the lagged climatic variables and the interaction between climatic variables. Moreover, the season effect is included in the model in order to assess the interaction between the season and climatic variables. In addition to climatic variables and season, other factors of the tree, which are diameter at breast height (DBH), radius and increment, were included in the model. It is important to note that the relationship between age and anatomical variables is not linear. Of all possible transformations, the square root of age is linearly related with the anatomical variables. Accordingly, throughout the analysis the square root of age is used instead of the age itself. Moreover, we assessed the effect of climatic variables on the wood anatomy using the daily average climatic measurements, obtained from dendrometer trial experiment. Therefore, our interest is to assess the rate of change of the wood anatomy for a unit change in the climatic variable.

Preliminary fitting of the model included the season, age, DBH, radius, increment and the climatic variables (including lags) effect. To choose the appropriate covariance structure, the model was fitted with ANTE (1), AR (1), ARH (1), ARMA (1, 1), CS, CSH, HF, TOEP, TOEPH, UN and VC covariance structures. From these covariance structures, the Compound symmetry (CS) and AR (1) were found to be the best covariance structures for between and within subject effects respectively. To choose the best covariance structure, we have used Akaikeis information criterion. The Akaikeis information criterion (AIC) is equal to -2 Res Log Likelihood plus twice the number of parameters in the covariance parameter structure model [21]. Here, AICC is the AIC corrected. It is the version of AIC which is adjusted for the effects of estimating parameters on the AIC itself [22]. BIC (Bayesian information criterion) is also based on -2 Res Log Likelihood. This value charges penalty when we have large number of parameters. The models were fitted using SAS PROC MIXED (ver. 9.1.3).

The first fibre and vessel characteristics to be considered is the fibre dimension (FD). The p-values for testing the significance of the effects in the final reduced model for Phase I is displayed in Tables 1. The significant effects, at the 0.05 level of significance, are explained below. The usual mixed model error assumptions for these models were checked using the residual plots. From the plots, it was observed that the usual model assumptions were not seriously violated by the data. As we can see from Table 1, the significant effects for GC and GU found to be different. This implies that we cannot have one model for the two clones. Table 1 shows the significant effects for FD for Phase I. After fitting the model, the observed and fitted values of FD for both clones are presented in Fig. 1. As we have seen in Fig. 1, the model for FD with the significant predictor variables fits well for both clones. The estimated values for the significant effects are presented in Table 1.

As we can see in Table 1, the effect of season for FD Phase I was found to be significant. This result indicates that the rate of increase for FD was found to be in summer (-4.35), autumn (-7.08) and winter (-6.80) as compared to spring (the reference season) for GC. This result implies that season has negative effect on FD. On the other hand, the effect of seasons found to be significant for GU. As the result indicates, FD was lower in autumn (-2.8) and winter (-1.79) as compared to spring for GU Phase I. But, for summer there was no significant effect.

One of the significant results in our model was the interaction between season and age. As we can see from the result, FD decreases with age in summer (-0.15), autumn (-0.02) and winter (-0.004) as compared to spring. On the other hand, FD decreases with age for autumn (-0.07) and winter (-0.03) as compared to spring for GU. But, similar to the season effect, when age interacts with summer, there was no significant effect.

From Table 1, we found that the interaction between temperature and season was found to be significant for autumn and winter for GC and GU respectively. FD decreases with temperature in autumn (-0.14) and increases in winter (0.307) for GC and GU respectively.

The other significant result in the model was the between solar radiation and season. The result was found to be significant for winter for GC and for autumn and winter for GU. As we can see from Table 1, FD increases with solar radiation in autumn (0.16) for GC. Similarly, FD for GU increases with solar radiation in autumn (0.13) and winter (0.15).

The other significant effects on FD for Phase I in the model were relative humidity and solar radiation interactions. This interaction effect found to be significant only for GC. From our result, we observed that the interaction between relative humidity and solar radiation has negative (-0.032) effect on FD for GC.

Table 1. Parameter estimates for FD Model: Phase I

Effect	GC			GU		
	Estimate	**SE**	**Pr >|t|**	**Estimate**	**SE**	**Pr >|t|**
Intercept	6.8748	0.4489	<.0001	3.6071	0.5133	0.0001
Sqtage	-0.4716	0.0318	<.0001	-0.2271	0.03552	0.0002
Summer	-4.3547	0.6584	<.0001	-0.5311	0.8104	0.5123
Autumn	-7.0766	0.393	<.0001	-2.9864	0.4899	<.0001
Winter	-6.7982	0.3952	<.0001	-1.7878	0.4892	0.0003
DBH	0.2505	0.02486	<.0001	0.09543	0.02653	0.0003
Increment	7.12E-06	1.83E-06	<.0001	2.76E-06	1.76E-06	0.1172
Temperature	0.05892	0.0381	0.1221	0.1214	0.04691	0.0097
Age*summer	0.2805	0.04112	<.0001	0.05532	0.05065	0.2748
Age *Autumn	0.4459	0.02737	<.0001	0.1848	0.034	<.0001
Age *winter	0.4679	0.02746	<.0001	0.1962	0.03397	<.0001
Temperature *summer	-0.0889	0.065	0.1713	-0.09	0.07984	0.2599
Temperature *autumn	-0.1371	0.04708	0.0036	-0.0754	0.05908	0.202
Temperature *winter	0.09148	0.05228	0.0802	0.1859	0.06437	0.0039
Solar radiation*autumn	0.07228	0.04698	0.124	0.1269	0.0599	0.0341
Solar radiation*winter	0.1576	0.04843	0.0011	0.146	0.05978	0.0146
Humidity*solar radiation	-0.032	0.01359	0.0186	-0.0178	0.01689	0.2933

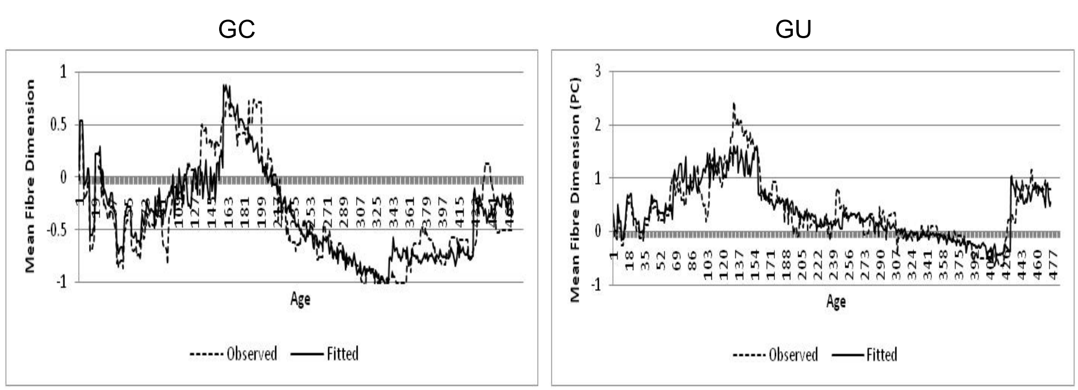

Fig. 1. Observed vs. fitted values for FD Phase I

The significant effects for Fibre Dimension (FD) for Phase II - IV for both clones show that the observed and fitted values of FD for both clones, the model for FD with the significant predictor variables fits well for both clones for Phase II - IV. The summarized results are presented as follows.

The combined effect of square root of age and season was found to be significant for Phase II and III. As the result indicates FD increases with age in summer for both clones for Phase II. Moreover, FD increases in winter with age for GC and in autumn for GU Phase III. The other significant effect for Phase II only was the combined effect of temperature and season. As can be seen from the result, FD was decreasing in winter for both clones for a change in temperature for Phase II. Furthermore, the combined effect of rainfall and season was found to be significant only for Phase II. As the result indicates, FD was increasing in winter for both clones for a change in rainfall. Similarly, the combined effect of the 16th day of solar radiation and season was found to be significant only for Phase III. As the result indicates FD increases in summer for a change in the 16th day of solar radiation. From the result, it was observed that there were no significant effects for Phase IV.

The results for the random effects for Phase I-IV are presented in Table 2. The table shows that the effect of tree was significant (p – value = 0.0453 for GU) but not significant for GC (p-value = 0.073) for Phase I. This result shows that there was variability from tree to tree for GC and GU for the change in mean FD. On the other hand, tree by square root of age interaction was significant (p-value = 0.0346) for GC but not for GU (p-value = 0.0942). Moreover, the result indicates that the slope of each tree was statistically different for GC clone. On the other hand, the estimated value 0.44 for GC and 0.60 for GU were found to be significant. These results indicate that the measurements between trees were different for FD Phase I.

On the other hand, the results for the random effects for Phase II to IV are presented in Table 2. As the result implies, there was tree to tree variability for Phase II and III. Nevertheless, there was no tree to tree variability for IV. Similarly, there was variability within trees for Phase II and IV. But, for Phase III there was no within subject (tree) effect. On the other hand, for Phase II GC, there was variability for the combined effect of tree and age.

The second fibre and vessel characteristic is the vessel dimension (VD). Table 3 shows the significant effects for VD for Phase I. From the table, it was observed that the significant effects for GC and GU found to be different. This implies that we cannot have one model for the two

Table 2. FD random effects variance test using continuous climatic variables

Cov parm	Subject	GC			GU		
		Estimate	**Z value**	**Pr Z**	**Estimate**	**Z value**	**Pr Z**
Phase I							
Variance	Treeno	0.0302	1.79	0.073	0.01607	1.69	0.0453
CS	Treeno	0.0278	1.82	0.0346	0.0149	1.67	0.0942
AR(1)	Treeno	0.4415	1.96	0.05	0.2659	1.83	0.0471
Residual		0.4028	45.89	<.0001	0.6042	44.44	<.0001
Phase II							
Variance	Treeno	0.1798	1.83	0.0429	0.2078	2.98	0.0498
CS	Treeno	0.1803	1.94	0.0452	0.1931	1.98	0.1382
AR(1)	Treeno	0.9807	156.47	<.0001	0.9378	107.66	<.0001
Residual		1.1978	3.09	0.001	0.7869	7.18	<.0001
Phase III							
Variance	Treeno	0.1798	1.83	0.0429	0.2078	1.98	0.0498
CS	Treeno	0.1803	1.94	0.0452	0.1931	1.98	0.0482
AR(1)	Treeno	0.9807	156.47	<.0001	0.9378	107.66	<.0001
Residual		1.1978	3.09	0.001	0.7869	7.18	<.0001
Phase IV							
Variance	Treeno	0.0052	0.69	0.2443	0	.	.
CS	Treeno	0.0031	0.56	0.5762	0.00095	1.38	0.1682
AR(1)	Treeno	0.5475	9.52	<.0001	0.4045	6.39	<.0001
Residual		0.2101	8.57	<.0001	0.3482	9.96	<.0001

Table 3. Parameter estimates for VD Model: Phase I

Effect	GC			GU		
	Estimate	**S E**	**Pr > |t|**	**Estimate**	**S E**	**Pr > |t|**
Intercept	1.4566	0.4956	0.0187	-0.6386	0.6977	0.3868
Summer	0.1215	0.6736	0.8569	1.4227	1.0883	0.1912
Autumn	-2.3228	0.398	<.0001	-1.1908	0.6539	0.0687
Winter	-2.1486	0.4044	<.0001	-1.1916	0.6559	0.0693
DBH	-0.407	0.02425	<.0001	0.1208	0.0347	0.0005
Radius	1.8E-05	7.34E-06	0.0134	4.5E-05	5.98E-06	<.0001
Temperature	0.0914	0.0386	0.018	0.0015	0.0626	0.0083
Rainfall	0.03339	0.0302	0.269	0.1125	0.04566	0.0138
Age*summer	0.00201	0.04207	0.9619	-0.0839	0.06803	0.2176
Age *autumn	0.1559	0.02776	<.0001	0.07475	0.0454	0.0998
Age *winter	0.1982	0.02829	<.0001	0.1113	0.04567	0.0148
Temperature *summer	-0.1006	0.06583	0.1266	-0.0531	0.1066	0.6184
Temperature *autumn	-0.199	0.0477	<.0001	-0.276	0.07892	0.0005
Temperature *winter	0.08825	0.05325	0.0975	-0.1792	0.08618	0.0376
Humidity*summer	-0.0455	0.05363	0.3963	-0.0423	0.08725	0.6278
Humidity *autumn	0.02097	0.04124	0.6111	-0.0458	0.06853	0.5042
Humidity *winter	0.2422	0.04041	<.0001	0.2003	0.06525	0.0022
Solar radiation*summer	-0.0043	0.04609	0.9265	0.01314	0.07434	0.8598
Solar radiation *autumn	0.06994	0.0477	0.1427	-0.1517	0.07993	0.0578
Solar radiation *winter	0.2077	0.04932	<.0001	0.1554	0.07987	0.0517
Temperature *wind speed	-0.0358	0.01555	0.0214	-0.0181	0.02592	0.4853
Temperature *solar radiation	-0.0298	0.01378	0.0307	-0.0123	0.02256	0.586

GC GU

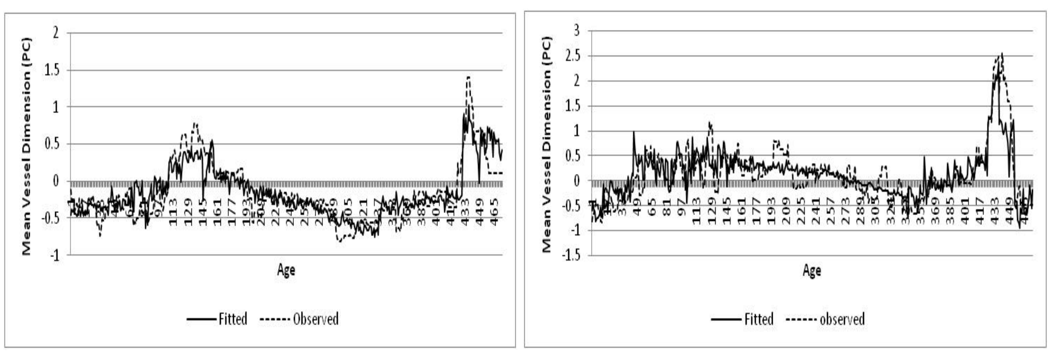

Fig. 2. Observed vs. fitted values for VD Phase I

clones. After fitting the model, the observed and fitted values of VD for both clones are presented in Fig. 2.

As we have seen in Fig. 2, the model for VD with the significant predictor variables fits well for both clones. The estimated values for the significant effects are presented in Table 3.

As we can see in Table 3, the effect of season for VD Phase I was found to be significant for GC. But, this effect was not significant for GU. This result indicates that VD was lower in autumn (-2.32) and winter (-2.15) as compared to spring (the reference season) for GC. This result implies that season has negative effect on VD for Phase I.

One of the significant results in our model was the interaction between season and age. As we can see from the result, VD increases with age in autumn (0.16) and winter (0.20) as compared to spring for GC. On the other hand, VD increases with age in winter (0.11) as compared to spring for GU.

From Table 3, we found that the interaction between temperature and season was found to be significant for both clones. As we can see from Table 3, VD decreases with the temperature in autumn (-0.11) for GC. On the other hand, VD decreases in autumn (-0.28) and winter (-0.18) as compared to spring for GU.

Similarly, from Table 3, we found that the interaction between relative humidity and season was found to be significant for both clones. VD increases in winter (0.24 and 0.20) for GC and GU respectively as compared to spring. As we can see from Table 3, the interaction between solar radiation and season was found to be significant for both clones. As we can see from Table 3, VD increases in winter (0.21 and 0.16) for GC and GU respectively as compared to spring. The other significant effects in the model were the interaction between temperature and wind speed, and the interaction between relative humidity and solar radiation for GC. But, these interaction effects were not significant for GU. The interaction effect between temperature and wind speed has negative (-0.036) effect on VD for GC. Similarly, the interaction between relative humidity and solar radiation has negative effect on VD for GC.

The significant effects for Vessel Dimension (VD) for Phase II - IV show that, the model for FD with the significant predictor variables fits well for both clones for Phase II - IV. The summarized results are presented as follows.

Similar to VD Phase I, the combined effect of square root of age and season was found to be significant for Phase II and III. As the result indicates, VD increases with age in autumn for both clones for Phase II. On the other hand, this combined effect for Phase III showed that VD decreases with age in summer and winter for GC and GU respectively. The other significant result in the model was the combined effect of temperature and season. This effect was significant only for Phase II. The result for Phase II shows that VD was VD was decreasing in summer for GC and increasing in winter for GU for a change in temperature for Phase II. The combined effect of season and relative humidity was found to be significant only for Phase III GU. As the result indicates, VD was increasing for all seasons for a change in relative humidity for Phase III GU. Moreover, the combined effect of solar radiation and season was found to be significant for Phase II GC only. As the result indicates VD increases in winter for a change in solar radiation for GC Phase II. The other significant effect in the model for Phase II was the combined effect of temperature and relative humidity for both clones and the combined effect of rainfall and solar radiation for GC only. As the result indicates, the combined effect of temperature and relative humidity has negative effect for both clones. Similarly, the combined effect of temperature and wind speed has negative effect on VD for GU Phase III. From the result, it was observed that there were no significant effects for Phase IV.

The random effect tests for VD for Phase I – IV are presented in Table 4. The table shows that the effect of tree was significant for GC. However, the tree effect was not significant for GU. This result shows that there was variability from tree to tree for GC for the change in mean VD. On the other hand, tree by square root of age interaction was not significant (p-value = 0.0668) for GC and (p - value = 0.1071) for GU. These results indicate that the slopes of each tree are not statistically different for both clones. On the other hand, the estimated value 0.95 for GC and 0.904 for GU were found to be significant. These results indicate that the measurements between trees were different for VD Phase I.

Furthermore, the results for the random effects for Phase II to IV are presented in Table 4. As the result for the random effects indicates, there was variability from tree to tree only for Phase II GU and Phase IV GC. For the rest phases, there was no variability between trees. Similarly, there was variability for the combined effect of age and tree for Phase IV GC. On the other hand, except for Phase III there was variability within trees for Phase II and IV.

The last fibre and vessel characteristic to be considered is the vessel frequency. Table 5 shows the significant effects for VF for Phase I. From the table, similar to the categorical climatic variables result, it was observed that the significant effects for GC and GU found to be different. This implies that we cannot have common model for the two clones. After fitting the model, the observed and fitted values of VF for both clones are presented in Fig. 3.

Table 4. VD random effects variance test using continuous climatic variable

Cov parm	Subject	GC			GU		
		Estimate	Z value	Pr Z	Estimate	Z value	Pr Z
Phase I							
Variance	Treeno	0.02967	1.85	0.0321	0.01598	1.63	0.0518
CS	Treeno	0.0273	1.83	0.0668	0.0148	1.61	0.1071
AR(1)	Treeno	0.9494	172.18	<.0001	0.9037	124.68	<.0001
Residual		0.5804	9.2	<.0001	1.2421	13.3	<.0001
Phase II							
Variance	Treeno	0.3681	1.81	0.0353	0.2172	1.89	0.0295
CS	Treeno	0.3556	1.81	0.0705	0.2084	1.89	0.0593
AR(1)	Treeno	0.9347	1.91	0.0698	0.561	1.98	0.0594
Residual		0.126	33.27	<.0001	0.2066	32.53	<.0001
Phase III							
Variance	Treeno	0.3681	1.81	0.0553	0.2172	1.89	0.0295
CS	Treeno	0.3556	1.81	0.0705	0.2084	1.89	0.0593
AR(1)	Treeno	0.1347	1.81	0.0698	0.0641	1.88	0.0594
Residual		0.126	33.27	<.0001	0.2066	32.53	<.0001
Phase IV							
Variance	Treeno	0.1004	1.52	0.0638	0.03612	0.77	0.2196
CS	Treeno	0.0823	1.51	0.1306	0.0272	0.7	0.4834
AR(1)	Treeno	0.3626	1.45	0.1484	0.173	0.85	0.3975
Residual		0.5751	12.58	<.0001	1.0934	12.59	<.0001

As we have seen in Fig. 3, the model for VF with the significant predictor variables fits well for both clones. The estimated values for the significant effects are presented in Table 5. As we can see in Table 5, the effect of season for VF Phase I was found to be significant for both clones. This result indicates that VF decreases in autumn (-1.49) and winter (-1.85) for GC and in summer (-3.5), autumn (-1.77) and winter (-1.996) for GU as compared to spring (the reference season). This result implies that season has negative effect on VF for both clones.

One of the significant results in our model was the interaction between season and age. As we can see from the result, VF increases with age in autumn (248.09) and winter (278.79) for GC. Similarly, VF increases with age in summer (886.01), autumn (286.82) and winter (308.45) for GU as compared to spring for Phase I.

From Table 5, we found that the interaction between temperature and season was found to be significant for both clones. VF increases with temperature in summer (0.019), autumn (0.077) and winter (0.053) for GC as compared to spring. On the other hand, VF increases with temperature in summer (0.031), autumn (0.035) and winter (0.0298) for GU as compared to spring for Phase I. The significant effects for Vessel Frequency (VF) for Phase II - IV for both clones show that the model for VF with the significant predictor variables fits well for both clones for Phase II - IV. The summarized results are presented as follows.

Similar to VD, the results for the random effects for Phase I - IV are presented in Table 6. The table shows that the effect of tree was not significant (p – value = 0.217) for GC and (p –value = 0.413) for GU. This result shows that there was no variability from tree to tree for both clones for a change in mean VF. On the other hand, tree by square root of age interaction was not significant (p-value = 0.719) for GC and (p – value = 0.141) for GU. These results indicates that the slope of each tree were not statistically different for both clones. On the other hand, the estimated value 0.92 for GC and 0.83 for GU were found to be significant. These results indicate that the measurements between trees were different for Phase VF I. As the result for the random effect indicates, there was tree to tree variation only for Phase II GC. For the other phases there was no variability between trees. Similarly, there was combined effect of reciprocal of age and tree for Phase II GC only. There was variability between trees with age. On the other hand, there was variability within trees for all phases except for Phase IV.

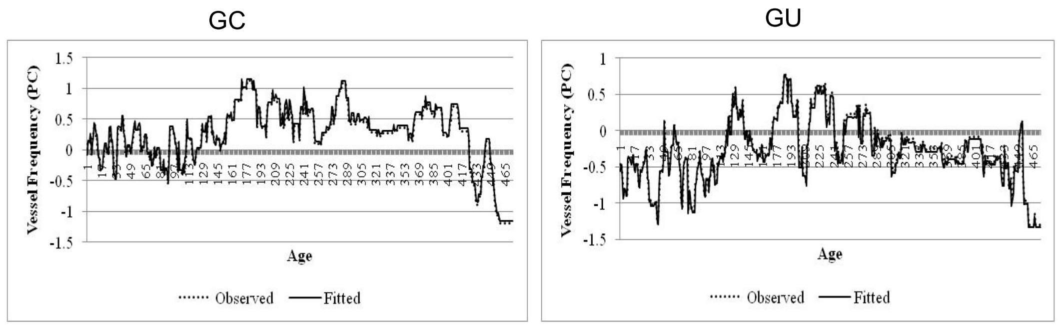

Fig. 3. Observed vs. fitted values for VF Phase I

Table 5. Parameter estimates for VF Model: Phase I

Effect	GC			GU		
	Estimate	S E	Pr > |t|	Estimate	S E	Pr > |t|
Summer	-2.6484	1.349	0.0613	-3.5025	1.2123	0.0081
Autumn	-1.4894	0.6724	0.0365	-1.7704	0.6081	0.0077
Winter	-1.8511	0.6952	0.0136	-1.9962	0.6305	0.0042
Humidity	0.01109	0.01066	0.298	0.0263	0.01185	0.0265
DBH	0.3008	0.03025	<.0001	0.1617	0.02873	<.0001
Radius	2.06E-07	4.04E-06	0.9593	0.00001	3.18E-06	0.0011
Age*summer	656.65	347.78	0.0591	886.01	314.2	0.0048
Age *autumn	248.09	123.41	0.0445	286.82	114.55	0.0123
Age *winter	278.79	125.06	0.0259	308.45	116.21	0.008
Temperature*summer	0.0191	0.04989	0.0118	0.0314	0.05401	0.0211
Temperature *autumn	0.0769	0.03531	0.0277	0.0351	0.04042	0.0354
Temperature *winter	0.05337	0.03464	0.0234	0.0298	0.03745	0.0464

Table 6. VF random effects variance test using continuous climatic variables

Cov parm	Subject	GC			GU		
		Estimate	Z value	Pr Z	Estimate	Z value	Pr Z
Phase I							
Variance	Treeno	0.6046	0.78	0.2165	0.03728	0.22	0.4132
CS	Treeno	0.0079	0.36	0.7192	0.01203	1.47	0.1412
AR(1)	Treeno	0.8964	127.67	<.0001	0.8667	108	<.0001
Residual		0.9219	14.79	<.0001	0.831	16.61	<.0001
Phase II							
Variance	Treeno	0.7241	1.8	0.0359	0	.	.
CS	Treeno	0.00345	1.88	0.0303	0.00099	1.26	0.209
AR(1)	Treeno	0.3897	1.98	0.0482	0.9778	239.15	<.0001
Residual		9.7851	33.37	<.0001	0.9587	5.44	<.0001
Phase III							
Variance	Treeno	0	.	0.2632	0	.	.
CS	Treeno	0.04002	1.12	<.0001	0.01997	0.93	0.3548
AR(1)	Treeno	0.9486	151.18	<.0001	0.9042	116.23	<.0001
Residual		0.6751	8.21		1.1632	12.37	<.0001
Phase IV							
Variance	Treeno	0.2918	1.47	0.071	0.1004	1.52	0.0638
CS	Treeno	0.0325	1.46	0.0726	-0.0823	-1.51	0.1306
AR(1)	Treeno	0.06885	0.96	0.3385	0.07155	1.02	0.3088

4. DISCUSSION

In this paper, the longitudinal linear mixed model with age, season, temperature, rainfall, solar radiation, relative humidity, wind speed and lagged climate variables as fixed effect factors and tree as random effect factor was fitted to the data. Besides the age, season and climatic variables (including lags) to improve the model, we included the dominance or suppression of the tree, the tree radius and daily radial increment in the model. To classify the trees as dominant and suppressed, the diameter at breast height (DBH) of the tree was used. The difference between the dominant and suppressed is that some trees are growing faster compared to other and this could affect the characteristics of the wood formed. To determine the dominance of the trees, the mean and the standard deviation of DBH of all trees in the plot was calculated. The trees were classified as dominant if the tree DBH was greater than mean plus one standard deviation, suppressed if it was less than mean minus one standard deviation and neither suppressed nor dominant if the DBH is within one standard deviation of the average.

The model selection process for fixed effects interaction was started by removing the insignificant highest order interaction effects from the full model then refitting the reduced model. This process continued until the final reduced model was obtained. Accordingly the highest order interactions in the model were three factor interactions. The interaction between tree and age was regarded as random effect.

Choosing the appropriate covariance structure is very important. To choose the best covariance structure, we have used likelihood based information criteria: The AIC, AICC and BIC. From the available different covariance structures, the compound symmetry and Autoregressive order 1 (AR (1)) were found to be the best covariance structures for between and within subject effects respectively.

From the fitted model analysis, the only common effect for FW, FD, VD and VF was found to be the joint effect of the square root of age and season. This means that the rate of increase/decrease of FW, FD, VD and VF against the square of age differed from season to season. For instance, for Phase I the GC clone FW increases in autumn but the GU clone FW increases in summer and autumn. On the other hand, FD decreases with age for both clones and VD increases in winter with age for both clones for Phase I. On the contrary VF increases with age in summer and autumn for both clones for Phase I. The joint effect of two climatic variables on FW, FD, VD and VF were different for each phase and each clone. For example for phase I, the joint effect of season and rainfall, season and solar radiation, season and wind speed, season and temperature at lag13, and solar radiation and wind speed were significant for GC clone FW. On the other hand, the significant joint effects for GU clone FW were relative humidity and wind speed, temperature at lag13 and season, and rainfall and season. Similarly, for Phase I GC clone FD, the significant joint effects were season and rainfall, season and relative humidity, season and solar radiation, rainfall and relative humidity and temperature and solar radiation. On the other hand, the significant joint effects for GU clone FD were season and solar radiation, and temperature and solar radiation.

The results of the random effects in the mixed model show that there was significant tree to tree FW variability for Phase I, Phase II GU and Phase III GU. But there was no significant tree to tree VD variability for Phase I, Phase II GC clone and Phase IV GC clone. Similarly, there was significant tree to tree FD and VF variability for Phases II and III. Moreover, there was a significant tree by age interaction effects for FW, FD and VD for all the phases, which shows that the slopes (the rate of daily increase/decrease) of FW, FD and VD of each tree were statistically different. But, for VF the slopes were identical for each tree.

In general, the two clones have different models for all fibre and vessel characteristics. The GC clone has more significant explanatory variables than the GU clone. Moreover, the fibre and vessel characteristics have different significant factors. For example, FW was affected by DBH and daily radial increment, VD was affected by the radius size for GC. From the four fibre and vessel characteristics VD and FD were affected by more variables than FW and VF. The only common significant factor for GC clone fibre and vessel characteristics was found to be the interaction between square root of age and season. But for the GU clone wood anatomy the only common significant factors were found to be the square root of age by season interaction and DBH. Generally summer and autumn are found to be the best seasons to produce larger fibre and vessel characteristics of the two *Eucalyptus* clones.

5. CONCLUSION

The wood anatomy characteristics for the two clones were affected by climatic variables when the tree was on Juvenile stage. But as the tree matures it might withstand with any climatic condition of the Zululand. This was supported by none existence of any significant climatic effects for Phase IV analyses. Moreover, the number of significant two-way interaction between climatic variables decreases as the phase increases. This might show that at the juvenile stage a combination of appropriate climatic conditions is more useful than the effect of a single climatic condition. But as the tree matures the joint effect of climatic variables combinations is minimal.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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