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APPLICATION OF ADDITIVE MIXED EFFECTS MODELS TO STUDY THE STEM RADIAL GROWTH OF EUCALYPTUS TREE

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Abstract: *Classical techniques such as simple and multiple parametric regression analyses assume the linearity of the relationship between the response and independent variables. Moreover, these classical techniques rely on the rigid assumptions of constant variance and the independent and identical distributions of the error terms. Consequently, a consideration of a more flexible approach can greatly influence the accuracy of our analysis. The objectives of this study are to evaluate and suggest an alternative approach to the classical parametric methods. The stem radius data obtained from Sappi landholdings in eastern South Africa were used. Stem radius of two hybrid clones, namely Eucalyptus grandis × Eucalyptus urophylla and E. grandis × E. Camaldulensis clone was used as the response variable. Additive mixed effects model that incorporates a non-parametric smooth function is used. Different additive mixed models were fitted to show the functional relationship between stem radius and tree age. The relationship between stem radius and tree age depends on clone and season. This study suggests semi-parametric approach as an alternative to the usual parametric approaches especially when the functional relationship between the response and the covariate is not known.*

Key words: *Additive Mixed effects, dendrometer trial, parametric modelling, penalized splines.*

1. Introduction

Statistical methods like normal regression models, the logistic regression model for binary data and Cox's proportional hazards model for survival data assume a linear, or some parametric form, for the covariate effects. However, in several applications, this assumption of linear dependence of the response on the predictors is not appropriate. Some authors reviewed and fitted stem radius data using parametric regression methods for longitudinal data [16], [17], [18], [19], [20], [21]. These parametric models provide a powerful tool for modelling the relationship between the responses and the covariates. However, parametric

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models suffer from inflexibility in modelling complicated relationships between the responses and covariates. In parametric methods, the form of the underlying relationship must be known in advance except for the values of a finite number of parameters. That means the relationship between the mean of the longitudinal response and the covariates is fully parametric.

The main drawback of parametric modelling is that it may be too restrictive or limited for many practical cases. This limitation has motivated a demand for developing non-parametric regression methods for analysis of longitudinal data. These methods can help to estimate a more flexible functional form between the responses and the covariates in the data. As a result, complicated relationships between longitudinal responses and covariates can possibly be captured from the data. The main idea behind the non-parametric approach is to let the data decide the most suitable form of the functions. According to Wu and Zhang 2006 [30] non-parametric and parametric regression methods should not be regarded as competitors, instead they complement each other. In some situations, non-parametric techniques can be used to validate or suggest a parametric model. A combination of both non-parametric and parametric methods is more powerful than any single method in many practical applications.

Although parametric models may be restrictive for some applications, non-parametric models may be too flexible to make concise conclusions in comparison with parsimonious parametric models. Semi-parametric models are good compromises and retain nice features of both the parametric and non-parametric models [4]. Significant changes in non-parametric and semi-parametric regression methods for longitudinal data have taken place in the past 20 years. The presence of the within-subject correlation among repeated measures over time presents major challenges in developing kernel and spline smoothing methods for longitudinal data [14]. As a result, the extension of classical local likelihood based kernel methods and their natural local estimating equation fails to account for the within-subject correlation. This leads to the development of a non-local kernel estimator. Some advanced kernel and spline-based methods for longitudinal data, have been developed recently. One such method is the extension of spline smoothing to longitudinal data. This extension entails clearly accounting for the within-subject correlation in building the penalized likelihood function. In this paper, the focus is on a class of splines referred to as penalized splines. Three motivational reasons for focusing on penalized splines are: (1) they are direct extensions of linear models (2) they are closely connected with linear mixed models and (3) their mixed model representation makes their extension to the longitudinal setting relatively straightforward.

A very flexible semi-parametric regression approach using the linear mixed model representation of penalized splines is described by Ruppert et al., 2003 [23]. The generalized additive models [9] are among those widely used non-parametric methods for independent data. The generalized additive models (GAM) can be represented using penalized regression splines. GAM with continuous response is called additive models. Additive models replace the linear relationship between the response and covariates to a relationship between the response and sum of smooth functions of covariates.

Regarding GAM, the works of Hastie and Tibshirani ([9], [12]), Faraway [5] and Wood ([27], [29]) can be cited as examples of good references. The underlying assumption on GAM is that the data are independent, which is not the case for longitudinal data. The extended form of GAM is called the generalized additive mixed model (GAMM). A GAMM with a Gaussian response is called additive mixed model (AMM). The aim of this paper is to review AMM and fit them to stem radius data. The objectives of this study were to develop semi-parametric linear mixed model for stem radius of two Eucalyptus *(E. grandis × E. urophylla and E. grandis × E. camaldulensis)* hybrid clones and compare their growth potential by comparing the estimates for each clone.

2. Materials and Methods

2.1. Data

A dendrometer trial focused on the growth of *an Eucalyptus grandis × E. urophylla (GU) and an E. gradis × E. camaldulensis (GC)* hybrid clones, and the trial established on Sappi landholdings near the town of KwaMbonambi, in the KwaZulu-Natal province of South Africa [16], [17], [18], [19], [20], [21]. The trial was designed to run over at least nine years with separate growth monitoring phases. Several wood characteristics are measured, and a huge database is acquired from the trial. The data used in this study are based on the data collected from April 2002 when trees were 39 weeks–old until August 2003 when trees were 107 weeks old. Using dendrometers repeated measurements of stem radius were obtained, during this time, for a sample of 18 trees, nine from each clone. Nine trees per plot were selected from each clone for intensive monitoring of radial growth [2], [3], [16], [17], [18], [19], [20], [21].

From the 18 sampled trees (nine per clone), longitudinal data of 1242 weekly stem radial measurements were obtained. The response variable investigated in this study was the weekly stem radial growth, which is of interest because it can be used to understand the underlying processes of fiber development in fast-growing *Eucalyptus* plantations. In addition, the study of young trees may be very important in the selection of a more productive tree species. Some studies have been made from the data extracted from the same Sappi data base. These are the study by Drew et al. [3] and studies by Melesse and Zewotir [16], [17], [18], [19], [20], [21]. One can refer to these studies for details of data collection, field preparations and soil survey. Some of these studies have considered the longitudinal nature of the data. However, these studies considered the parametric modelling approach and none of these studies considered the non-parametric or semi-parametric approaches.

2.2. Methods

The classical linear regression model and cross-sectional study only deal with the average change and does not provide any information about how individuals change over time. The detection of changes in the characteristics of the target population at both group and individual level can only be achieved within a longitudinal study. A distinguishing feature of longitudinal data is that observations within the same individual are correlated. It is important to account for the effect of correlation to avoid an erroneous estimation of the variability of parameter estimates. This interdependence can be modelled using mixed models.

The current data set consisted of repeated measurements of the same subjects over time. Numerous linear and nonlinear mixed-effects have been proposed [6], [22], [25], [26] in the analysis of the longitudinal data. Models for the analysis of such data recognize the relationship between serial observations on the same unit. Most of the work on methods of repeated measures data has focused on data that can be modelled by an expectation function that are either linear or non-linear in its parameters [13], [18]. However, none of these studies considered the non-parametric functions in their model. The main objective of this study is to show the application of semi-parametric (additive mixed models) to the stem radius data described above.

The additive model can be formulated by admitting the smooth function of some predictor variables in the classical linear regression model.

$$y = X^{*}\alpha + \sum_{j=1}^{p} f_j\left(x_j\right) + \varepsilon; \qquad \varepsilon \sim N(0, \sigma^2 I) \tag{1}$$

where: X^{*} is a model matrix for the parametric components of the model, α is the corresponding parameter vector and the $f_j(\,.\,)$ is a smooth arbitrary function of a covariate x_j, ε is the vector of random errors. The assumptions of the additive model are the same as the assumptions in the linear model except for the assumption of linear relationship between the response and covariates. These are homoscedasticity, the error variance is the same whatever is the value of the explanatory variable, the error is normally distributed, and the errors are uncorrelated. The inclusion of the random effects into the additive model (1) gives us the additive mixed model.

$$y = X^{*}\alpha + \sum_{j=1}^{p} f_j\left(x_j\right) + Zb + \varepsilon; \tag{2}$$

where: *Z* is the design matrix for random effects *b*. ε is a vector of random error which is independent of *b*.

$$\varepsilon \sim N(0, R)$$

and

$$b \sim N(0, G_{\theta}).$$

Both R and G_θ are positive definite covariance matrices. These matrices are also assumed to depend on a parsimonious set of covariance parameters. The AMM that can have non-normal response is the GAMM. A GAMM has the following structure.

$$G(y) = X^{*}\alpha + \sum_{j=1}^{p} f_j\left(x_j\right) + Zb + \varepsilon \tag{3}$$

where: G (.) is a monotonic differentiable function. A GAMM represents the model with higher flexibility and complexity, where mixed effects, smooth terms and non-normal responses are included [15]. These models can be viewed as additive extensions of the generalized linear mixed models.

Statistical inference in generalized additive mixed models comprises estimations of the non-parametric functions $f_j(.)$, the smoothing parameters, λ, and all the variance components. In the case of Gaussian response and identity link function, the estimation of non-parametric functions, smoothing and variance parameters in the context of GAMM is achieved using Restricted Maximum Likelihood (REML).

For non-Gaussian response, PQL (Penalized Quasi Likelihood) [1] and DPQL (Double Penalized Quasi Likelihood) are used to estimate the parameters and non-parametric function [15]. Both PQL and DPQL take their origin from maximum likelihood (ML) technique. The ML has direct application only in fixed models with Gaussian response. The maximum likelihood approach is also used in linear mixed models; however, the maximum likelihood estimators (MLE) of variance are, in general, biased. As a result, REML estimators are used instead of maximum likelihood estimators.

Both ML and REML assume that the response is normally distributed. The assumption of normality is often easily violated in practice making the likelihood inference difficult. In the absence of the random effects and errors distributions, the likelihood function cannot be available. Even in the presence of non-normal distributions of the random effects and errors with some unknown parameters, the likelihood function can involve quite formidable difficulty in calculation and may not have an analytic appearance. Moreover, the distributional assumptions for any non-normal distribution may not hold in practice. These problems have led to the attention of methods other than maximum likelihood. One such method is the quasi-likelihood also known as Gaussian likelihood approach. The computational difficulty of the maximum likelihood method can be avoided by using quasi-likelihood. The REML estimates can be derived from a quasi-likelihood [11]. Therefore, the Gaussian REML estimation can be considered as a method of quasi-likelihood.

When the exact likelihood function is computationally intractable, there are no simple solutions to get the parameter estimates. One viable option is to use numerical integration techniques. Some of these are Gaussian quadrature, numerical integration like Markov chain, Monte Carlo algorithms, stochastic approximations algorithms and penalized quasi-likelihood [31]. Penalized likelihood estimation has been proposed as a computationally simple alternative to methods based on numerical quadrature, especially when the number of random effects is relatively large [6]. The key concept in quasi-likelihood is Laplace approximation. For details of Laplace approximation one can refer to [6], [8], [15], [24], [31].

The Software for GAMM -although several R packages (R core team, 2013) are developed to fit GAMM, the most versatile that can handle modelling the correlation structure is the package mgcv [28]. This uses the nlme implementation of nonlinear mixed models. It also fits non-Gaussian responses by calling *MASS's* generalized linear mixed model penalized quasi-likelihood (glmmPQL). The main advantage of this package is that it is possible to include serial and/or spatial correlation structures of the random effects. In this paper, the package mgcv [28] is used to fit the additive mixed models.

3. Results and Discussions

At the beginning the AMM that involves only tree age as an explanatory variable is considered. The estimated smoothed curve together with its 95% confidence interval is shown in Figure 1.

Figure 1 indicates that the relationship between stem radius and tree age is nonlinear. In this plot the stem radius is expressed in mean deviation form, the smooth terms $s_j(x_j)$, where x_j stands for the covariate tree age is centred and hence the plot represents how stem radius change relative to its mean, with change in covariate under consideration.

The interpretation of the scale of the graph is as follows: A plot of the variable age versus the smooth term, $s(\text{Age})$, shows the relationship between tree age and stem radius. However, the stem radius is expressed in mean deviation form. Therefore, the smooth term, $s(\text{Age})$, is also centred and thus each plot represents how stem radius changes relative its mean with change in age. Hence, the value of zero on the vertical axis is the mean of stem radius. As the line moves away from zero in a negative direction we subtract the distance from the mean to determine the fitted value. If the line moves in a positive direction, we add a similar distance. For instance, to get the fitted value for stem radius when tree age is 46 weeks, we need to add the value the smooth term, $(s(\text{Age}))$ corresponding to age is equal to 46 (-10000) in Figure 1 and the mean radius (16240.27). That means the fitted value when tree age is 46 weeks is equal to 6240.27. The fitted value will be around 21240 micro meters when the tree age is about 90 weeks.

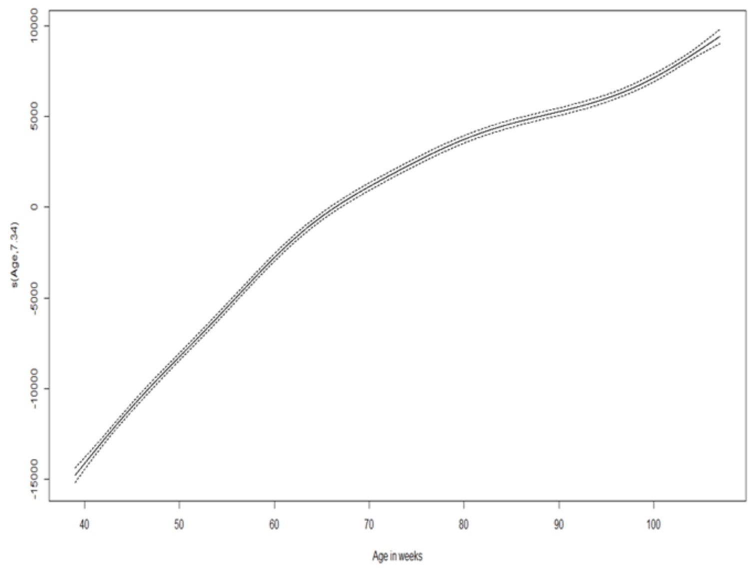

Fig. 1. *Estimated smoothing curve for the simplest AMM model (the solid line is the smoother and the dotted lines are 95% confidence intervals)*

The non-linearity was tested using a formal test by comparing a model specifying the smooth term with a model specifying a linear trend. The difference between the two models (linear trend versus smooth terms) is statistically significant (*p-value less than 0.0001*). Moreover, the estimated effective degree of freedom is 7.3 confirming the non-linearity of the relationship. This indicates the inclusion of the non-parametric part is important. The effect of tree age on stem radius may vary with clone or season. Instead of applying one smoother for both clones, a model with two smoothers (one smoother for each clone) is fitted to study the effect of tree age on stem radius. The model with clone added is better judged by likelihood ratio test statistics (255.7, *df*=2 and *p-value < 0.0001*). Therefore, a model with one smoother per clone is preferable to the model with one smoother for both clones. The results of the fitted additive mixed model with two different smoothers (one per clone) are presented in Figure 2 and Table 1.

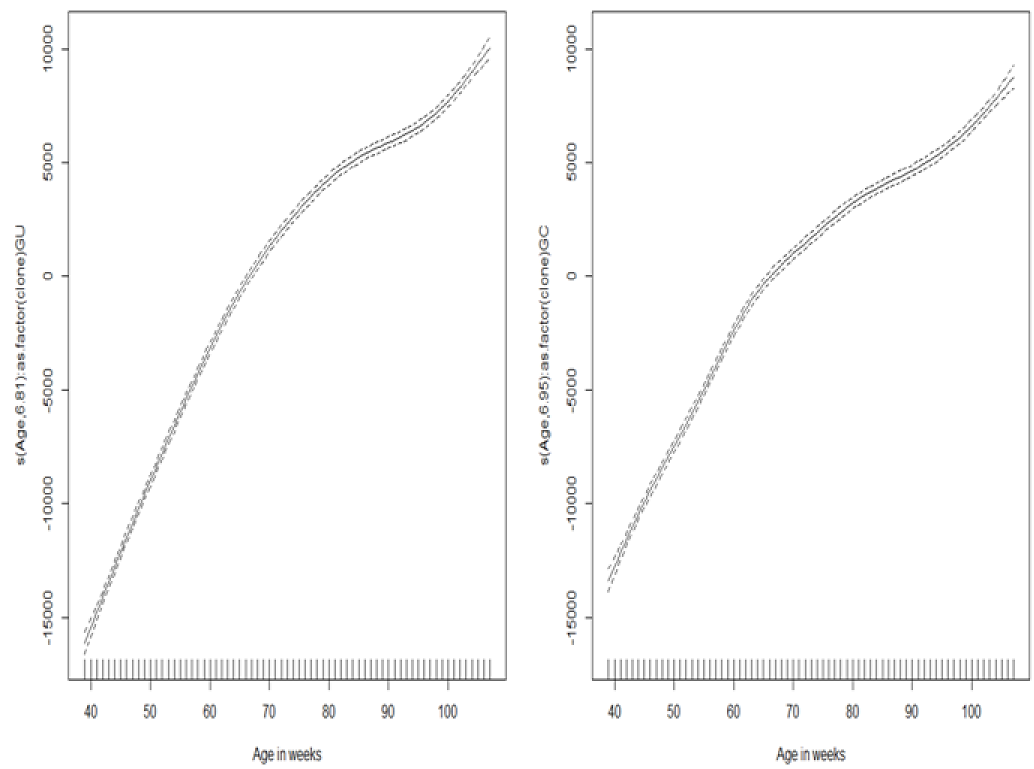

Fig. 2. *Estimated smoothing curve for the GAMM model that uses tree age by clone as an explanatory variable (the solid line is the smoother and the dashed lines are 95% confidence intervals)*

The effect of tree age is estimated as smooth curves with 6.806 and 6.954 effective degrees of freedom for GU and GC clones respectively. The *p-values* for both smoothed terms are very small (*p-value < 0.0001*) and very large value of *F* with corresponding *p-value less than 0.0001* (Table 1). This indicates that the relationship between tree age and stem radius remains non-linear after adding the clone to the model. The adjusted R^2 (the square of the correlation coefficient between observed and fitted values) is 0.821. This shows that there is a strong relationship between observed and fitted values of the model.

The QQ plot and the histogram of residuals show some non-normality (Figure 3). The residuals versus predictor plot shows that there is a clear violation of homogeneity of variance. The plot of the response against fitted value shows that there is a strong linear relationship between the observed response and the fitted value. Before fitting more complicated models (e.g. additive mixed models with more complex covariance structure), an attempt to extend the current model with the effect of more than one covariate was made.

Table 1

The fitted additive mixed model with one smoother of tree age per clone (Maximum likelihood estimates)

Parametric coefficients	Estimate	Standard error	t-value	p-value
Intercept	16240.3	671.6	24.18	< 0.0001
Approximate significance of smooth terms				
	Edf	Ref. df	F-value	p-value
s(Age, clone=GU)	6.806	6.806	2925	< 0.0001
s(Age, clone=GC)	6.954	6.954	1951	< 0.0001
R-sq.(adj) = 0.821				

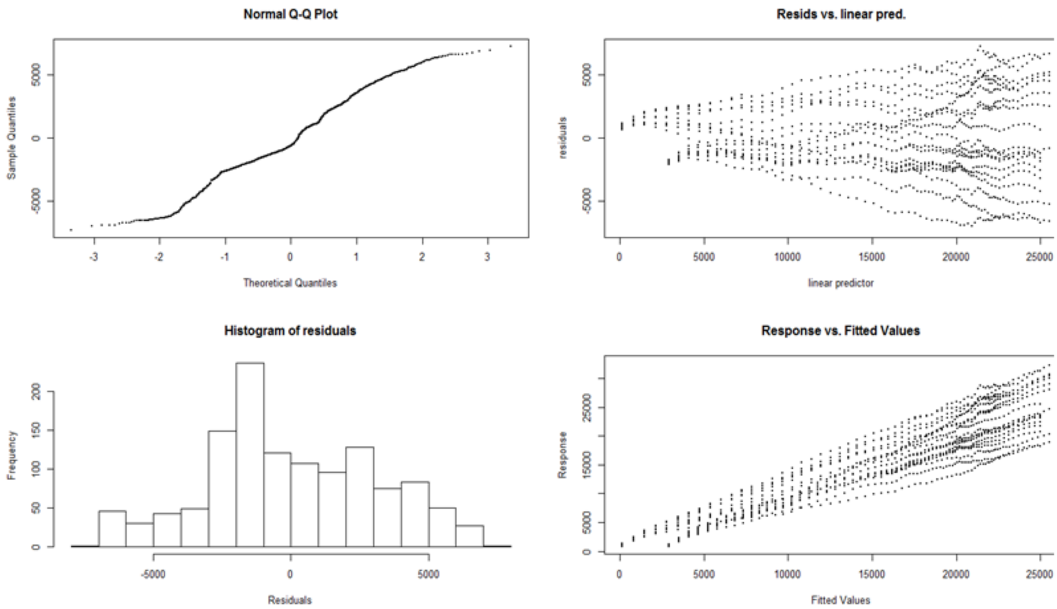

Fig. 3. *Model validation graphs for the additive mixed model that has two smooth curves of tree age (one per clone)*

An attempt to fit eight smoothers (one for each clone and season combination) was not successful due to numerical problems encountered. Instead a model with four smoothers for each clone is fitted after separating the data into two, namely the data for GU and the data GC clone. For GU clone, the smoothers for all seasons have very large values of *F* in each case with corresponding small (*p-values < 0.0001*). This indicates the relationship between tree age and stem radius appears to be nonlinear for all seasons with a slight variation in the values of effective degrees of freedom (edf) (Table 2).

For GC clone, the smoothers for all seasons are significant *(p < 0.0001)* (Table 3). This shows that the two clones grow in an analogous manner which means, in both cases, the relationship between tree age and stem radius is nonlinear and the non-parametric part of the model is highly significant. Moreover, the estimates for the intercept in Tables 3 and 4 indicate the estimates for parametric part of the model. The estimated intercept for GU clone is larger than that of GC. This shows that the GU hybrid is economically viable hybrid cross as reported elsewhere [7], [18], [19].

Table 2

The fitted additive mixed model with four different smoothers of tree age (one per season) for the GU clone (Maximum likelihood estimates)

Parametric coefficients	Estimate	Standard error	t-value	p-value
Intercept	17563	1073	16.37	< 0.0001
Approximate significance of smooth terms				
	Edf	Ref. df	F-value	p-value
s(Age, season = Summer)	2.162	2.162	103.45	< 0.0001
s(Age, season = Autumn)	3.541	3.541	2469.08	< 0.0001
s(Age, season = Winter)	3.286	3.286	1343.93	< 0.0001
s(Age, season = Spring)	2.183	2.183	53.16	< 0.0001
R-sq.(adj) = 0.818				

Table 3

The fitted additive mixed model with four different smoothers of tree age (one per season) for the GC clone (Maximum likelihood estimates)

Parametric coefficients	Estimate	Standard error	t-value	p-value
Intercept	15101.9	641.1	23.55	< 0.0001
Approximate significance of smooth terms				
	Edf	Ref. df	F-value	p-value
s(Age, season = summer)	2.086	2.086	79.98	< 0.0001
s(Age, season = Autumn)	3.888	3.888	3150.49	< 0.0001
s(Age, season = Winter)	3.886	3.886	1715.73	< 0.0001
s(Age, season = Spring)	2.092	2.092	48.44	< 0.0001
R-sq.(adj) = 0.899				

An attempt to fit the model with four smoothers of age (one for each season) was made by including the interaction between clone and season on the parametric part of the additive mixed model. The results of the model fit show that all parametric coefficients and the smooth terms are significant. For summer and spring the smoothers have an effective degree of freedom equal to one, essentially fitting a straight line (Table 4). This shows the relationship between stem radius and tree age is linear in summer and spring by considering the parametric and non-parametric effects of clone and season. Figure 4 also confirms that the type of relationship between stem radius and tree age depends on season.

Table 4

The fitted additive mixed model with four different smoothers of tree age (one per season) with the interaction between season and clone included in parametric part (Maximum likelihood estimates)

Parametric coefficients	Estimate	Standard error	t-value	p-value
Intercept	20338.8	868.0	23.43	< 0.0001
Clone (GC)	-3796.9	1194.7	-3.18	0.00152
Season (Autumn)	-3291.7	407.9	-8.07	< 0.0001
Season (Winter)	-2722.6	468.3	-5.81	< 0.0001
Season (Spring)	-652.4	299.9	-2.18	0.02982
Clone (GC) × Season (Autumn)	1478.2	233.2	6.34	< 0.0001
Clone (GC) × Season (Winter)	1327.5	239.4	5.54	< 0.0001
Clone (GC) × Season (Spring)	1025.0	263.6	3.89	0.00011
Approximate significance of smooth terms				
	Edf	Ref. df	F-value	p-value
s(Age, season = Summer)	1	1	70	< 0.0001
s(Age, season = Autumn)	3.321	3.321	4823.6	< 0.0001
s(Age, season = Winter)	3.307	3.307	2559.2	< 0.0001
s(Age, season = Spring)	1	1	175.4	< 0.0001
R-sq.(adj) = 0. 85				

The upper left and the lower right panels of Figure 4 show the relationship between tree age and stem radius is linear in both summer and spring. The upper right and the lower left panels of Figure 4 show the relationship between tree age and stem radius in autumn and winter respectively. It seems that the relationship is clearly nonlinear for autumn and winter. A similar model, but without the interaction effect of clone and season in the parametric part is fitted for comparison with the current model under consideration. The value of the likelihood ratio test statistic is 43.91 with 3 degrees of freedom and *p-value* *<0.0001*. Therefore, we cannot further simplify the model with the interaction of clone and season. The output for this model is presented in Table 4.

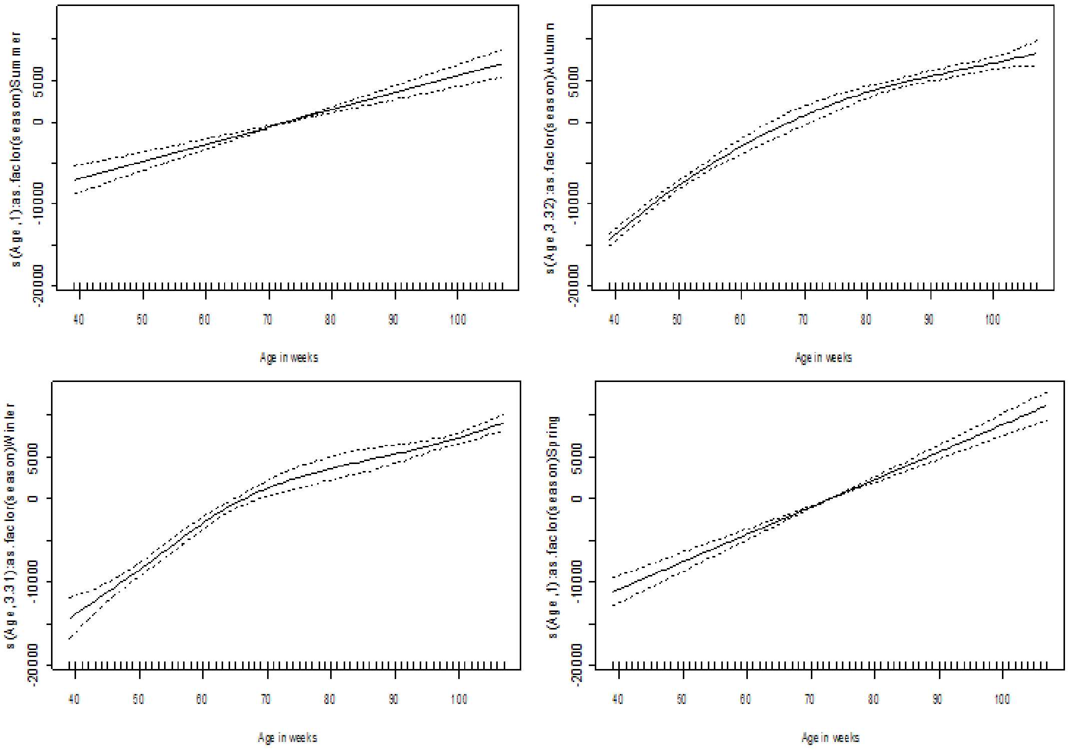

Fig. 4. *Estimated smoothing curves and 95% confidence bands for the GAMM model that uses four smoothers of tree age and includes the interaction of season by clone in the parametric part*

For all parametric methods, the form of the underlying relationship between the response and the covariates must be known in advance. Only a few numbers of parameters must be estimated to get the relationship between the response and covariates. The semi-parametric methods can provide a chance for the underlying relationships to be estimated in a data driven way. That means the type of relationship between the variables is decided by the data rather than by intuitions.

4. Conclusion

The semi-parametric models are introduced and applied. It was found that the relationship between stem radius and tree age can be better explained by a nonlinear relationship. The effect of tree age on stem radius varies with season. The adjusted R^2 used as a measure of the relationship between the observed and fitted values shows the relationship between observed and fitted stem radius is the strongest (R^2=0.82). In summary, the conclusions made in the semi-parametric methods agree with that of the parametric methods. Moreover, the semi-parametric approaches can help to describe the relationship between the response and the covariates in a data driven way. In the absence of known functional relationship between the response and covariate the semi-parametric methods are a more appropriate choice to model the stem radial growth.

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