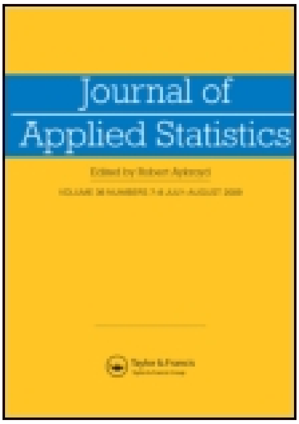

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Many-to-one comparison of nonlinear growth curves for Washington's Red Delicious apple

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Many-to-one comparison of nonlinear growth curves for Washington's Red Delicious apple

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In this article, we are interested in comparing growth curves for the Red Delicious apple in several locations to that of a reference site. Although such multiple comparisons are common for linear models, statistical techniques for nonlinear models are not prolific. We theoretically derive a test statistic, considering the issues of sample size and design points. Under equal sample sizes and same design points, our test statistic is based on the maximum of an equi-correlated multivariate chi-square distribution. Under unequal sample sizes and design points, we derive a general correlation structure, and then utilize the multivariate normal distribution to numerically compute critical points for the maximum of the multivariate chi-square. We apply this statistical technique to compare the growth of Red Delicious apples at six locations to a reference site in the state of Washington in 2009. Finally, we perform simulations to verify the performance of our proposed procedure for Type I error and marginal power. Our proposed method performs well in regard to both.

Keywords: multiple comparisons to control; multivariate chi-square distribution; nonlinear growth curves; Richards' curve; simulated critical points

1. Introduction

The state of Washington is a leading agricultural state and grows over half of the apples produced in the USA annually. Of all the apples it produces, Washington is most recognized for its Red Delicious apple. It is one of the big export crops for the state of Washington. It has long been of interest of growers and horticulturists to understand the growth pattern of apples. As a result, the Washington Tree Fruit Research Commission is collecting data over the next three years to model the growth of Washington's Red Delicious apple. The data set used in this manuscript was collected between May and August 2009. The data were collected at a reference site[1] (Naches) and six other research stations across the state (Omak, Chelan, Orondo, Wenatchee, Royal City,

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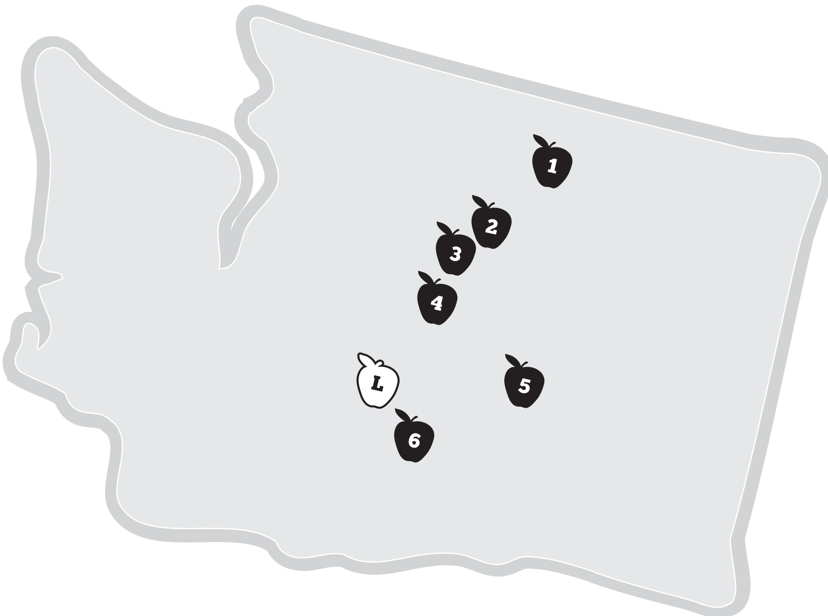

Figure 1. Multiple comparison of growth: are there differences in growth patterns at the six research stations (labeled 1–6) compared to the reference cite (labeled L)?

and Wapato), see Figure 1. One of the initial questions asked was: is there a difference in the growth pattern for the six sites to that of the reference site?

Apple growth has been studied extensively using several different units of analysis and many different types of models. Studies in photosynthesis [4], the root stock [1,23,29,44], the tree growth itself [37], the fruit load on tree architecture [9,23], and the entire orchard system [20] all relate to the final output of apple sizes and yields [42]. There have been temperature studies [22] relating to the importance of rest [6,7], blossoming [15,16] of fruit buds [26] with early-season temperatures [2], and other temperature measures [39]. Understanding final sizes and yields are complicated because there are so many different ways to model the growth of the apple, and depending on the practices at a given orchard, there may be variability in outcomes.

Our data are comprised of repeated diameter measurements from bloom to harvest for a sample of Red Delicious apples at the seven different locations. Research has shown that this diameter depends on cell count, volume, and density [8,43]. Diameter growth, in general, has been described as a sigmoidal or double sigmoid growth curve [25].

2. Statistical precedence

The study of growth in the biological sciences is the foundation of mathematical statistics. From the studies of crop variation by Fisher [see 24], to the human characteristic studies of Pearson [30,31], most statistical techniques have been developed to understand and study growth. Early work on growth curves was very descriptive in nature focusing on cross-tabular comparisons of key variables such as age, gender, and height on weight [18]. Potthoff and Roy [32] developed a generalized multivariate analysis of the variance model to solve basic "growth curve problems" including tests to compare the growth of two groups (males and females) using "*m*"-parameter polynomial models and the expectation of the growth outcome(s). This generalized form has been demonstrated to be very robust [3,14,27,32,33,40,41], and relies on F-tests to determine the order of the polynomial to consider and to compare an unrestricted effects-model (e.g. gender effect, could be multivariate) to a restricted model (all parameters are equal to some common vector of parameters). Such analysis addresses fundamental aspects of multiple comparison [13,21,28]; however, there is a need for more sophisticated multiple comparison techniques to compare groups simultaneously.

The literature comparing growth curves is not as prolific. Royen [36] discussed a method for comparing several polynomials. Heckman and Zemar [19] described a non-parametric technique that addresses shapes and groups simultaneously. Dasgupta *et al.* [12] focused on comparing several logistic regression curves. Another heuristic multiple-comparison approach has been developed by Bretz *et al.* [5]. They describe a technique to search among various generalized models (mostly nonlinear) to identify a best model that explains optimal dosages across treatments while controlling for "family-wise error rates" using critical values simulated from a multivariate t-distribution [17].

3. Our contribution

In this article, we introduce a general multiple comparison technique for nonlinear models. Specific to the study of apple growth, we apply this technique to a three-parameter variation of the nonlinear model known as the Richards' curve [35], yet we derive a test statistic that can be applied to any k-parameter nonlinear model. Similary, we emphasize that we apply this technique specifically to compare six locations to a reference site; however, this technique can be applied to any $L-1$ multiple comparisons (locations, treatments, etc.) to a referent. Additionally, we provide methods of calculating the test statistic based on the number of actual observations at any particular site. In the real world, the execution of a design is not always ideal, as circumstances prevent simultaneous measurement, apples fall off the tree, etc. Our approach accounts for such a reality.

4. Model specification

Many sigmoid growth functions are available to model apple growth. In this research, we choose to utilize a three-parameter variation of the Richards' curve [35] to model apple growth:

$$Y_{ji} = f(X_{ji}; \underset{\sim}{\Theta}_i) = f(X_{ji}; \beta_i, \delta_i, \tau_i) = \frac{\beta_i}{(1 + \mathrm{e}^{-\delta_i(X_{ji}-\tau_i)})}, \tag{1}$$

where Y_{ji} represents the growth of the apple's (j) diameter (in inches) for location i at time X_{ji}.

Generally, the nonlinear model is fit using various observations at specific points in time (X_{ji}) for a given location. For example, 100 apples may be measured at six different points in time to give a total sample size of 600 observations for a specific location. Another location, however, may sample 100 apples measured at nine design points, and these nine points may or may not be different that the six design points for the first location. We describe this phenomenon in this form $X_{n_j,s}$ where n_j is the number of observations at a given design point and s is the number of design points. From this, the number of observations for a location is defined as $N_i = \sum_{j=1}^{s} n_j$. The last two columns of Table 1 describe details regarding the design points (Julian days when data are collected) and number of fruit collected at each site.

We choose this model for several reasons. First, the parameters have conceptual meaning to the apple growers: the maximum apple size (β), its growth rate (δ), and the time of maximum growth (τ). Second, this three-parameter model assumes: that at time $X_{ji} = 0$, the apple's size is zero; growth is symmetric around its inflection point ($X_{ji} = \tau_i$); the carrying capacity represents the maximum apple size; and this model form would allow for accommodating other factors by including more parameters. Third, this specific variation of the Richards' curve is a reasonably behaved[2] nonlinear model.

Based on our model selection, the parameter vector Θ for each location i is

$$\underset{\sim}{\Theta}_i = \begin{pmatrix} \beta_i \\ \delta_i \\ \tau_i \end{pmatrix}, \tag{2}$$

which defines a $k = 3$ parameter model.

We estimate our parameter vector using the Gauss–Newton method to get our least square estimate vector for each location as $\hat{\underset{\sim}{\Theta}}_i$. We define the $\text{var}(\hat{\underset{\sim}{\Theta}}_i) = \Sigma_i$. The form of Σ_i depends upon the method[3] of implementation for computing the nonlinear model. For this data, our Σ_i is defined as

$$\Sigma_i = \sigma^2 (D_i' D_i)^{-1}, \tag{3}$$

where D_i is the matrix of partial first derivatives of Equation (1) with respect to the parameters $\underset{\sim}{\Theta}_i$

$$D_i = \begin{bmatrix} b_{1i} & d_{1i} & t_{1i} \\ b_{2i} & d_{2i} & t_{2i} \\ b_{3i} & d_{3i} & t_{3i} \\ \vdots & \vdots & \vdots \\ b_{N_i i} & d_{N_i i} & t_{N_i i} \end{bmatrix}, \quad \text{where} \quad \begin{aligned} b_{ji} &= \frac{\partial Y_i}{\partial \beta_i} = \frac{1}{1 + \mathrm{e}^{-\delta_i (X_{ji} - \tau_i)}}, \\ d_{ji} &= \frac{\partial Y_i}{\partial \delta_i} = \frac{-\beta_i (X_{ji} - \tau_i)\, \mathrm{e}^{-\delta_i (X_{ji} - \tau_i)}}{[1 + \mathrm{e}^{-\delta_i (X_{ji} - \tau_i)}]^2}, \\ t_{ji} &= \frac{\partial Y_i}{\partial \tau_i} = \frac{-\beta_i \delta_i\, \mathrm{e}^{-\delta_i (X_{ji} - \tau_i)}}{[1 + \mathrm{e}^{-\delta_i (X_{ji} - \tau_i)}]^2}. \end{aligned} \tag{4}$$

Hence,

$$D_i' D_i = \begin{bmatrix} \sum_{j=1}^{N_i} b_{ji}^2 & \sum_{j=1}^{N_i} b_{ji} d_{ji} & \sum_{j=1}^{N_i} b_{ji} t_{ji} \\ & \sum_{j=1}^{N_i} d_{ji}^2 & \sum_{j=1}^{N_i} d_{ji} t_{ji} \\ & & \sum_{j=1}^{N_i} t_{ji}^2 \end{bmatrix}. \tag{5}$$

In Table 1, we report the model fit for the parameters at each location and the corresponding variance–covariance structure of the parameter estimates.

5. Hypothesis, test statistic, and critical values

The formal hypothesis is that all of the comparison sites are equivalent to the reference site, with the alternative being at least one is different from the reference site. We outline a test that is based on both the modeled parameters for each location and the corresponding variance–covariance structure:

$$\begin{aligned} H_0 &: \underset{\sim}{\Theta}_i = \underset{\sim}{\Theta}_L \quad \forall\, i = 1, 2, \ldots, L-1, \\ H_a &: \underset{\sim}{\Theta}_i \neq \underset{\sim}{\Theta}_L \quad \exists\, i = 1, 2, \ldots, L-1. \end{aligned} \tag{6}$$

From Equations (3)–(5), we identify that Σ_i depends on: the design points X_{ji}, the sample size N_i, and the parameter values. Hence, to address statistical considerations with this form of the covariance matrix, we enumerate three cases: (Case I) when the sample sizes for the treatments (e.g. comparison sites) are equal; (Case Ia) when the sample sizes for the treatments are equal but unequal from the control (e.g. reference site); and (Case II) when the sample sizes for the treatments are unequal.

Table 1. Data results: locations, parameter estimates, and design frame.

		Estimates			Variance–covariance				Design frame	
City	Location	β	δ	τ		β	δ	τ	X_{ij} design points	n_{ij} replicates
Omak	1	3.1818	0.0323	182.1612	β	0.0007450	–	–	170, 181, 195, 212, 231, 245, 260	100, 100, 100, 99, 99, 98, 95
					δ	−0.0000201	0.0000007	–		
					τ	0.0145313	−0.0003492	0.3565604		
Chelan	2	3.0472	0.0357	169.9224	β	0.0008285	–	–	169, 181, 195, 212, 245, 260	100, 99, 99, 96, 94, 87
					δ	−0.0000361	0.0000022	–		
					τ	0.0092310	−0.0001266	0.3700854		
Orondo	3	3.3683	0.0369	171.4220	β	0.0013617	–	–	169, 195, 212, 231, 245, 260	100, 100, 87, 95, 97, 92
					δ	−0.0000478	0.0000021	–		
					τ	0.0173506	−0.0003319	0.6112461		
Wenatchee	4	3.4746	0.0244	176.0775	β	0.0046068	–	–	166, 216, 229, 245, 260	100, 99, 100, 97, 87
					δ	−0.0000747	0.0000014	–		
					τ	0.1136279	−0.0016730	3.2668883		
Royal City	5	3.1347	0.0382	172.2522	β	0.0012414	–	–	177, 195, 201, 232, 258, 260	100, 100, 100, 99, 98, 98
					δ	−0.0000741	0.0000062	–		
					τ	0.0092310	0.0008309	0.7809501		
Wapato	6	3.1961	0.0333	170.7713	β	0.0021051	–	–	189, 195, 201, 218, 230, 246, 268	100, 99, 98, 95, 95, 91, 89
					δ	−0.0001115	0.0000071	–		
					τ	−0.017678	0.0020210	1.3785176		
Naches	L	2.8705	0.0345	173.0170	β	0.0006904	–	–		
					δ	−0.0000454	0.0000038	–	189, 195, 201, 212, 218, 226, 233,	100, 100, 97, 97, 94, 95, 73,
					τ	−0.0089750	0.0013170	0.9556438	244, 251, 257, 264, 271, 278	63, 62, 63, 61, 61, 56

Case I: Same design points and equal sample sizes. Under the null hypothesis as described in Equation (6), $\Sigma_1 = \Sigma_2 = \cdots = \Sigma_{L-1} = \Sigma_L$ iff $X_{ji} = X_j \ \forall\ i$ and $N_1 = N_2 = \cdots = N_{L-1} = N_L$. Therefore, for Case I,

$$\Sigma_1 = \Sigma_2 = \cdots = \Sigma_{L-1} = \Sigma_L. \tag{7}$$

Here, using the multivariate form of the central limit theorem, recalling that $\Sigma_i = \Sigma$ is a function of $N_i = N$ derived from Equations (3)–(5), we know that

$$\lim_{N\to\infty} (\hat{\Theta}_i - \Theta_i) \xrightarrow{\mathrm{d}} N(0, \Sigma) \tag{8}$$

and

$$\begin{pmatrix} (\hat{\Theta}_1 - \Theta_1) \\ (\hat{\Theta}_2 - \Theta_2) \\ \vdots \\ (\hat{\Theta}_{L-1} - \Theta_{L-1}) \\ (\hat{\Theta}_L - \Theta_L) \end{pmatrix} \xrightarrow{\mathrm{d}} N\left(\begin{pmatrix} \mathbf{0} \\ \mathbf{0} \\ \vdots \\ \mathbf{0} \\ \mathbf{0} \end{pmatrix}, \begin{pmatrix} \Sigma & 0 & 0 & \cdots & 0 & 0 \\ 0 & \Sigma & 0 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & \Sigma & 0 \\ 0 & 0 & 0 & \cdots & 0 & \Sigma \end{pmatrix} \right); \tag{9}$$

therefore, under the null hypothesis

$$\begin{pmatrix} (\hat{\Theta}_1 - \hat{\Theta}_L) \\ (\hat{\Theta}_2 - \hat{\Theta}_L) \\ \vdots \\ (\hat{\Theta}_{L-2} - \hat{\Theta}_L) \\ (\hat{\Theta}_{L-1} - \hat{\Theta}_L) \end{pmatrix} \xrightarrow{\mathrm{d}} N\left(\begin{pmatrix} \mathbf{0} \\ \mathbf{0} \\ \vdots \\ \mathbf{0} \\ \mathbf{0} \end{pmatrix}, \begin{pmatrix} 2\Sigma & \Sigma & \Sigma & \cdots & \Sigma & \Sigma \\ \Sigma & 2\Sigma & \Sigma & \cdots & \Sigma & \Sigma \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ \Sigma & \Sigma & \Sigma & \cdots & 2\Sigma & \Sigma \\ \Sigma & \Sigma & \Sigma & \cdots & \Sigma & 2\Sigma \end{pmatrix} \right), \tag{10}$$

where the variance–covariance matrix in Equation (10) can be written as

$$\begin{bmatrix} H & \rho H & \rho H & \cdots & \rho H \\ \rho H & H & \rho H & \cdots & \rho H \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \rho H & \rho H & \rho H & \cdots & H \end{bmatrix}, \tag{11}$$

where $H = 2\Sigma$ and $\rho = \frac{1}{2} I_{k\times k}$.

The resulting test statistic is defined as

$$M_U = \max(U_1, U_2, \ldots, U_{L-1}), \tag{12}$$

where

$$U_i = (\hat{\Theta}_i - \hat{\Theta}_L)' H^{-1} (\hat{\Theta}_i - \hat{\Theta}_L). \tag{13}$$

Since $(U_1, U_2, \ldots, U_{L-1})$ follow a multivariate chi-square distribution with dependence parameter ρ [11,36], the critical value c is defined as

$$P(M_U > c \mid H_0) = \alpha. \tag{14}$$

We use critical points based on the maximum order statistic of the multivariate chi-square. Appropriate tables have been defined by Dasgupta [10]; appropriate `R` code to generate critical values is available[4] from the authors.

Case Ia: Same design points and unequal sample sizes for control. Here, we have the same design points for all L groups $(X_1, X_2, \ldots, X_d)$. At each design point (X_j), n_i observations are allocated to the $(L-1)$ treatment groups and m_i observations are allocated for the control group, L, such that $m_i = rn_i$.

$$\sum_{i=1}^{s} n_i = N, \tag{15}$$

$$\sum_{i=1}^{s} m_i = M, \tag{16}$$

where $M = rN$. In this situation, $D_i = D$ for $i = 1, 2, \ldots, L-1$ and $D_L = rD$. Hence,

$$\Sigma_L = \sigma^2 (D_L' D_L)^{-1} = r^{-1}(D'D)^{-1}\sigma^2 = \frac{1}{r}\Sigma. \tag{17}$$

Following the same logic as in Equations (8) and (9), Equation (10) updates to

$$\begin{pmatrix} (\hat{\Theta}_1 - \hat{\Theta}_L) \\ (\hat{\Theta}_2 - \hat{\Theta}_L) \\ \vdots \\ (\hat{\Theta}_{L-2} - \hat{\Theta}_L) \\ (\hat{\Theta}_{L-1} - \hat{\Theta}_L) \end{pmatrix} \xrightarrow{\text{d}} N\left(\begin{pmatrix} \mathbf{0} \\ \mathbf{0} \\ \vdots \\ \mathbf{0} \\ \mathbf{0} \end{pmatrix}, \begin{pmatrix} \Sigma_L + \Sigma & \Sigma_L & \Sigma_L & \cdots & \Sigma_L & \Sigma_L \\ \Sigma_L & \Sigma_L + \Sigma & \Sigma_L & \cdots & \Sigma_L & \Sigma_L \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ \Sigma_L & \Sigma_L & \Sigma_L & \cdots & \Sigma_L + \Sigma & \Sigma_L \\ \Sigma_L & \Sigma_L & \Sigma_L & \cdots & \Sigma_L & \Sigma_L + \Sigma \end{pmatrix}\right), \tag{18}$$

and the variance–covariance matrix has the same form described in Equation (11). As a result, the test statistic and critical points are derived in the same manner where now $H = ((1+r)/r)\Sigma$ and $\rho = (1/(1+r))I_{k\times k}$.

Case II: Different design points and unequal sample sizes (most general case). Here, we consider the situation similar to our apple data where we have unequal sample sizes and/or design points. This is the most general case. From Equation (3), it is evident that if $X_{ji} \neq X_j$ for all locations $i = 1, 2, \ldots, L-1, L$, or if $N_i \neq N$ for all locations $i = 1, 2, \ldots, L-1, L$ then Σ_i which depends on X_{ji} and N_i through D_i will not be the same under the null hypothesis. Again, following the logic of Equations (8) and (9), Equation (18) updates to

$$\begin{pmatrix} (\hat{\Theta}_1 - \hat{\Theta}_L) \\ (\hat{\Theta}_2 - \hat{\Theta}_L) \\ \vdots \\ (\hat{\Theta}_{L-2} - \hat{\Theta}_L) \\ (\hat{\Theta}_{L-1} - \hat{\Theta}_L) \end{pmatrix} \xrightarrow{\text{d}} N\left(\begin{pmatrix} \mathbf{0} \\ \mathbf{0} \\ \vdots \\ \mathbf{0} \\ \mathbf{0} \end{pmatrix}, \begin{pmatrix} \Sigma_L + \Sigma_1 & \Sigma_L & \Sigma_L & \cdots & \Sigma_L & \Sigma_L \\ \Sigma_L & \Sigma_L + \Sigma_2 & \Sigma_L & \cdots & \Sigma_L & \Sigma_L \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ \Sigma_L & \Sigma_L & \Sigma_L & \cdots & \Sigma_L + \Sigma_{L-2} & \Sigma_L \\ \Sigma_L & \Sigma_L & \Sigma_L & \cdots & \Sigma_L & \Sigma_L + \Sigma_{L-1} \end{pmatrix}\right). \tag{19}$$

Writing the variance–covariance matrix as in Equation (10), we now defined H most generally as $H_{ii} = \Sigma_i + \Sigma_L$; the dependence parameter ρ now becomes a dependence matrix R. The test statistic for this case is defined as

$$M_V = \max(V_1, V_2, \ldots, V_{L-1}), \tag{20}$$

where

$$V_i = (\hat{\Theta}_i - \hat{\Theta}_L)' H_{ii}^{-1} (\hat{\Theta}_i - \hat{\Theta}_L). \tag{21}$$

Table 2. Simulated quantiles for actual sample design frame.

Quantile	90%	91%	92%	93%	94%	95%	96%	97%	98%	99%
Critical value	9.7620	10.0258	10.3008	10.6205	10.9884	11.4354	11.8970	12.5694	13.4774	15.0404

Now $(V_1, V_2, \ldots, V_{L-1})$ follow a multivariate chi-square distribution with dependence matrix R. To delineate the form of R, we need to define B_i such that $B_i' B_i = \Sigma_i + \Sigma_L$. Hence,

$$\begin{bmatrix} I & R_{21} & R_{31} & \cdots & R_{(L-2)1} & R_{(L-1)1} \\ R_{12} & I & R_{32} & \cdots & R_{(L-2)2} & R_{(L-1)2} \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ R_{1(L-2)} & R_{2(L-2)} & R_{3(L-2)} & \cdots & I & R_{(L-1)(L-2)} \\ R_{1(L-1)} & R_{2(L-1)} & R_{3(L-1)} & \cdots & R_{(L-2)(L-1)} & I \end{bmatrix}, \tag{22}$$

where $R_{ii} = \Sigma_L (B_i B_i')^{-1}$.

To find critical values based on the maximum of $(V_1, V_2, \ldots, V_{L-1})$, we need to know the structure of matrix R. As no analytical form is immediately available, we simulate critical values using the multivariate variance–covariance structure for our apple data found in Table 1. This structure is defined based on the sample sizes[5] of each location. Table 2 shows the simulated (run 100,000 times) for our specific data example (with unequal sample sizes).

For $\alpha = 0.05$, we identify 11.435436 as the critical point specific to the number of locations and the unique design frames at each location.

6. Application of technique

We begin by estimating $\hat{\Sigma}$ which Dasgupta [10] demonstrated was a consistent estimator for Σ. In this case, we pool the variance across all locations based on the actual sample size from each location:

$$\hat{\Sigma} = \frac{\sum_{i=1}^{L} (N_i - 1) \hat{\Sigma}_i}{(\sum_{i=1}^{L} N_i) - L}. \tag{23}$$

In addition, we also consider $\hat{\Sigma}_L$ as an alternate estimator for Σ. The nature of our apple data is based on unequal sample sizes and design points, so we proceed utilizing[6] Case II, calculating H_{ii} for each location.

Whether we use the pooled variance or the control variance as the estimator for Σ with our apple data, we reject the null hypothesis and conclude that at least one location's growth curve is different from the control growth curve, see Table 3. Identifying the maximum, we can conclude that apple growth at Orondo (3) is statistically different from the apple growth at Naches (L). The built in multiple comparison allows us to also conclude that all locations other than Omak are different from Naches.

7. Simulations for size and power

In the earlier sections, we provide a method for comparing nonlinear curves to that of a control. Our test statistic asymptotically follows the maximum of a multivariate chi-square distribution. To understand its performance for finite samples, we run a Monte Carlo simulation for Type I error and power under the cases presented in this research. Recall, that the non-centrality parameter for

Table 3. Application of multiple comparison to control.

City	Location	V_i Control $\hat{\Sigma}_L$ $\hat{H}_{ii} = \hat{\Sigma}_i + \hat{\Sigma}_L$	V_i Pooled $\hat{\Sigma}$ $\hat{H}_{ii} = \hat{\Sigma}_i + \hat{\Sigma}$
Omak	1	3.54	2.38
Chelan	2	12.85	9.38
Orondo	3	58.94	39.97
Wenatchee	4	11.74	10.26
Royal City	5	25.07	16.04
Wapato	6	27.27	18.70
M_V as MAX		58.94	39.97
Simulated critical value		11.4354	
Hypothesis inference		Reject H_0	

any location in the multivariate chi-square distribution is defined as

$$\text{ncp} = (\Theta_i - \Theta_L)' H_{ii}^{-1} (\Theta_i - \Theta_L). \tag{24}$$

Case I: Same design points and equal sample sizes. For Type I error, we generate data under the null and look at the effect of sample size and error variance on the Type I error. Here we generate date from $(L-1) = 6$ locations and a control with $\beta_1 = \beta_2 = \cdots = \beta_L = 2.87$, $\delta_1 = \delta_2 = \cdots = \delta_L = 0.0345$, $\tau_1 = \tau_2 = \cdots = \tau_L = 173$. We chose these values as these are the estimated values for our reference site (Table 1). We varied σ^2 from 0.05 to 0.25 in increments of 0.05, as our estimated variance from our data was 0.15. We used the same design points $\{X_1, X_2, \ldots, X_6\} = \{170, 188, 206, 242, 280\}$ for all six locations and the control to span our data range. We then varied the sample sizes from 10 to 200 in our simulations and calculated the Type I error. Table 4 shows the results. Our table entries are the number of hypothesis rejections out of the total number of "good simulations". We defined a "good simulation" as the case when the nonlinear estimator (nls) converged for all six treatments and a control. If the nls estimator failed to converge even for one of the locations, we considered it a "bad" simulation. Our results indicate that the Type I error was controlled well even for sample sizes around 30 with all the Type I errors being within 2 standard deviations of the simulation error ($=\sqrt{(0.05)(0.95)/10{,}000} = 0.0021$). However, we see for smaller sample sizes, we have a big number of "bad simulations", which increases with the error variance. As a matter of fact, when $n_{ij} = 10$ and $\sigma^2 = 0.25$ only 1028 cases out of the 10,000 are "good" simulations. Meaning in 90% of the cases the estimates from the nls for one of the locations did not converge. For a sample size of 50, 87% of the cases were "good" simulations for the same error variance. This indicates that the Type I error performance depends on the convergence of the nls estimator. The nls estimator seems to converge well when sample sizes are around 50 with our six design points.

For marginal power [38], fixing $\sigma^2 = 0.15$, we generate data as described above for all but one location ($i = 2, 3, \ldots, L-1$), and we vary the parameter values for one location ($i = 1$). Based on the context of our data and results, we vary the parameters for Omak (1) in the following ranges: $\beta[2.57, 3.20]$ in 0.07 increments; $\delta[0.0258, 0.0420]$ in 0.0017 increments; and $\tau[167, 185]$ in 2 increments. In turn, we vary each parameter in isolation (fixing the other parameters at the predefined values for the Type I simulations). We also have cases of simulations where we varied all three parameters together. This is available from the authors and is not included for space considerations. The case with $\beta = 2.87$, $\delta = 0.0345$, and $\tau = 173$ corresponds to the null case. In Tables 5, we summarize the results. We again report the number of hypothesis rejections out of the total number of "good simulations". The results indicate that our method is quite sensitive

Table 4. Type I error, Case I: For $\alpha = 0.05$, nsim=10,000, and reasonable variances for Y, all locations are assigned these true parameters at the design points based on the sample sizes, with random noise (var(Y)).

var(Y)	$n_{ij} = 10$	$n_{ij} = 30$	$n_{ij} = 50$	$n_{ij} = 100$	$n_{ij} = 200$
0.05	$924/8351 = 0.11060$	$723/9989 = 0.07238$	$667/10,000 = 0.06670$	$602/10,000 = 0.06020$	$582/10,000 = 0.05820$
0.10	$329/4587 = 0.07172$	$776/9612 = 0.08073$	$665/9974 = 0.06667$	$640/10,000 = 0.06400$	$605/10,000 = 0.06050$
0.15	$114/2400 = 0.04750$	$646/8754 = 0.07380$	$720/9780 = 0.07362$	$653/9996 = 0.06533$	$603/10,000 = 0.06030$
0.20	$45/1496 = 0.03008$	$527/7504 = 0.07023$	$748/9330 = 0.08017$	$665/9965 = 0.06673$	$574/10,000 = 0.05740$
0.25	$33/1028 = 0.03210$	$406/6421 = 0.06323$	$680/8776 = 0.07748$	$661/9907 = 0.06672$	$635/9999 = 0.06351$

Notes: We report the number of rejects (using the pooled variance, see Equations (14) and (23)) based on good simulations (e.g. simulations for which the Gauss–Newton technique for nonlinear fitting converges, etc.) for six fixed design points $X_{ij} = \{170, 188, 206, 224, 242, 260\}$. Under the null, parameters at location L represent the true parameters: $\beta = 2.87$, $\delta = 0.0345$, and $\tau = 173$.

Table 5. Marginal power – changing one parameter at one location.

Parameters			Case I, location L_1					Case III	
$\beta = 2.87$	$\delta = 0.0345$	$\tau = 173$	$n_{ij} = 10$	$n_{ij} = 30$	$n_{ij} = 50$	$n_{ij} = 100$	$n_{ij} = 200$	Location L_1	Location L_6
	Type I error		114/2400 = 0.04750	646/8754 = 0.07380	720/9780 = 0.07362	653/9996 = 0.06533	603/10,000 = 0.06030	488/10,000 = 0.04880	438/10,000 = 0.04380
2.57			56/2405 = 0.0233	854/8560 = 0.0998	2347/9719 = 0.2415	6708/9990 = 0.6715	9883/10,000 = 0.9883	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
2.64			45/2353 = 0.0191	576/8529 = 0.0675	1247/9736 = 0.1281	3938/9994 = 0.3940	8650/10,000 = 0.8650	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
2.71			54/2437 = 0.0222	383/8607 = 0.0445	642/9784 = 0.0656	1625/9995 = 0.1626	4564/10,000 = 0.4564	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
2.78			44/2461 = 0.0179	294/8655 = 0.0340	357/9782 = 0.0365	509/9996 = 0.0509	1150/10,000 = 0.1150	10,000/10,000 = 1.0000	9997/10,000 = 0.9997
2.85			37/2528 = 0.0146	215/8702 = 0.0247	251/9797 = 0.0256	158/9996 = 0.0158	209/10,000 = 0.0209	1438/10,000 = 0.1438	769/10,000 = 0.0769
2.92			51/2534 = 0.0201	219/8649 = 0.0253	180/9768 = 0.0184	144/9995 = 0.0144	144/10,000 = 0.0144	9473/10,000 = 0.9473	7382/10,000 = 0.7382
2.99			61/2566 = 0.0238	210/8716 = 0.0241	217/9816 = 0.0221	318/9994 = 0.0318	1149/10,000 = 0.1149	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
3.06			54/2608 = 0.0207	279/8781 = 0.0318	387/9794 = 0.0395	1272/9998 = 0.1272	5211/10,000 = 0.5211	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
3.13			66/2583 = 0.0256	352/8769 = 0.0401	853/9787 = 0.0872	3707/9995 = 0.3709	9082/10,000 = 0.9082	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
3.20			66/2632 = 0.0251	548/8763 = 0.0625	1904/9813 = 0.1940	6969/9996 = 0.6972	9943/10,000 = 0.9943	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
	0.0258		42/2162 = 0.0194	394/8067 = 0.0488	783/9430 = 0.0830	1803/9939 = 0.1814	5066/9995 = 0.5069	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
	0.0276		41/2239 = 0.0183	352/8243 = 0.0427	533/9617 = 0.0554	1069/9973 = 0.1072	2814/10,000 = 0.2814	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
	0.0294		36/2276 = 0.0158	309/8345 = 0.0370	411/9667 = 0.0425	632/9982 = 0.0633	1360/10,000 = 0.1360	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
	0.0312		51/2389 = 0.0213	259/8532 = 0.0304	340/9707 = 0.0350	351/9994 = 0.0351	559/10,000 = 0.0559	9986/10,000 = 0.9986	9670/10,000 = 0.9670
	0.0330		40/2418 = 0.0165	241/8686 = 0.0277	270/9747 = 0.0277	220/9993 = 0.0220	254/10,000 = 0.0254	3862/10,000 = 0.3862	2289/10,000 = 0.2289
	0.0348		45/2481 = 0.0181	190/8662 = 0.0219	205/9777 = 0.0210	142/9994 = 0.0142	150/10,000 = 0.0150	148/10,000 = 0.0148	128/10,000 = 0.0128
	0.0366		46/2543 = 0.0181	203/8725 = 0.0233	203/9792 = 0.0207	110/9999 = 0.0110	114/10,000 = 0.0114	7765/10,000 = 0.7765	5365/10,000 = 0.5365
	0.0384		49/2603 = 0.0188	182/8808 = 0.0207	157/9819 = 0.0160	117/9993 = 0.0117	194/10,000 = 0.0194	10,000/10,000 = 1.0000	9959/10,000 = 0.9959
	0.0402		45/2566 = 0.0175	170/8820 = 0.0193	150/9798 = 0.0153	145/9995 = 0.0145	430/10,000 = 0.0430	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
	0.0420		40/2626 = 0.0152	174/8789 = 0.0198	152/9794 = 0.0155	233/9997 = 0.0233	905/10,000 = 0.0905	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
		167	44/2487 = 0.0177	236/8608 = 0.0274	336/9759 = 0.0344	676/9990 = 0.0677	2157/10,000 = 0.2157	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
		169	49/2429 = 0.0202	203/8605 = 0.0236	246/9769 = 0.0252	315/9995 = 0.0315	721/10,000 = 0.0721	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
		171	46/2478 = 0.0186	224/8668 = 0.0258	212/9793 = 0.0216	165/9996 = 0.0165	202/10,000 = 0.0202	7920/10,000 = 0.7920	7920/10,000 = 0.7920
		173	48/2491 = 0.0193	197/8654 = 0.0228	219/9773 = 0.0224	154/9995 = 0.0154	155/10,000 = 0.0155	105/10,000 = 0.0105	97/10,000 = 0.0097
		175	45/2552 = 0.0176	233/8652 = 0.0269	254/9806 = 0.0259	290/9996 = 0.0290	352/10,000 = 0.0352	7887/10,000 = 0.7887	7887/10,000 = 0.7887
		177	64/2613 = 0.0245	284/8731 = 0.0325	374/9789 = 0.0382	637/9994 = 0.0637	1294/10,000 = 0.1294	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
		179	43/2465 = 0.0174	397/8641 = 0.0459	621/9782 = 0.0635	1517/9993 = 0.1518	3610/10,000 = 0.3610	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
		181	58/2592 = 0.0224	537/8720 = 0.0616	1137/9787 = 0.1162	3202/9995 = 0.3204	6906/10,000 = 0.6906	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
		183	57/2632 = 0.0217	774/8804 = 0.0879	2075/9824 = 0.2112	5378/9994 = 0.5381	9116/10,000 = 0.9116	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000
		185	89/2603 = 0.0342	1169/8728 = 0.1339	3259/9761 = 0.3339	7643/9997 = 0.7645	9894/10,000 = 0.9894	10,000/10,000 = 1.0000	10,000/10,000 = 1.0000

to small departures from the null. This does depend upon the sample size which again highly influences the number of "good" simulations. For example, when we change β from 2.87 to 2.57, keeping δ and τ fixed at the reference values, the power changes from 0.02 for $n_{ij} = 10$ to 0.24 at sample size 50, to 0.99 for sample size 100. So, the results are not unexpected, as sample size increases so does the marginal power. As conceptually Case 1a is similar to Case 1, we do not include specific simulations for this case.

Case II: Different design points and unequal sample sizes (most general case). To conduct Monte Carlo simulations in Case II is daunting as there are infinite possibilities with different sample sizes and different design points even within the data range of our example. So we chose to simulate data choosing the sample size and the design points from our apple data. This allowed us to estimate the $H_{ii} = \Sigma_L + \Sigma_i$ and construct the overall covariance matrix as in Equation (18). Under the null, based on Equation (18), the differences of parameters to control is distributed as multivariate normal with mean zero and the covariance matrix as described based on $H_i = \Sigma_L + \Sigma_i$. Because the variance–covariance structures of each model are different (note: the sign differences in the off-diagonals), we simulate two locations. For each simulation at a location, we only vary the mean difference for that location, and test M_V using the simulated critical value reported in Table 2. In the last two columns of Table 5, we summarize the results. We see with the sample frame described in Table 1, the test is quite sensitive to small departures from the null case. Going from the reference value of 2.87 to 2.85 for β keeping δ and τ fixed gives a marginal power of 14.4%. Changing δ from 0.0345 to 0.0330 keeping the other two parameters fixed gives a power of 38.6%. Similarly changing τ from 173 to 175, with the others fixed gives a power of 79%. When changing all the three parameters together, we generally see full power (100%) for very small departures from the null. This sensitivity analysis of power further validates our proposed method.

8. Discussion and conclusion

In this paper, motivated by a problem of comparing growth curves, we introduce a method based on Wald statistics. Our test statistic as the maximum of the statistics at each location and is fairly easy to compute. We have an inbuilt follow-up which allows us to identify which locations are different than the control. We demonstrated how to estimate the H matrix (or specifically with our data, H_{ii}). We considered both a pooled variance $\hat{\Sigma}$ as an estimate for Σ and a control variance $\hat{\Sigma}_L$ as an alternative estimate for Σ. Theoretically our test statistic is the maximum of a multivariate chi-square distribution equi-correlated under equal sample sizes and identical design points with a general dependence matrix under unequal sample sizes and/or design points. We use methods in the literature to compute critical points for equal samples and use simulation methods to calculate the critical points for unequal sample sizes.

In this paper, we focus on the three-parameter Richards' curve to address our specific problem; however, we note that this technique is generalizable to other growth curves, and to other nonlinear forms. Our algorithm is flexible and the calculation of the critical points under unequal sample sizes makes it more applicable in practice. Hence, we foresee potential applications of this method in agricultural, biological, environmental, business, marketing, the social sciences, etc. In addition, future research can theoretically address pairwise comparison of curves and comparing curves to the "best" curve. In our example, we had a known reference site, Naches and it was of interest to compare the other sites to this. What if the reference site is not known? In such a scenario, "comparison to best" would make sense; however, this involves a nonlinear comparison and whether the procedure generalizes from our method for the vector of parameters remains to be seen. We plan on extending our results to this avenue in the future. Another avenue of future research is looking at comparing a large number of curves and using false discovery rates.

While, we propose a general technique, there were some issues specific to *our* problem that we faced and addressed. These issues are not universal and not theoretical, but we want to share our experiences with this problem as future researchers may face similar problems. The biggest problem we faced was related to the order of magnitude of our parameters. The β parameter which, in our model, implies the maximum growth of the fruit is less than 4 inches. Obviously, one does not encounter too many apples more than 4 inches in diameter in practice. The second parameter δ is the growth rate in the Richards' curve. This parameter is always less than 1 and generally quite small (between 0.02–0.04, in our data) as the rate of growth of a fruit is not expected to be large. The third parameter, τ represents the time of maximum growth and in Julian days is around 170–185 (around mid-year). These parameters make sense in the context of the problem and are of interest to growers. However, mathematically there are different orders of magnitude which makes off-diagonals of the variance–covariance matrix unique. This might lead to instability or "ill-conditioning" of the covariance matrix which may have, in turn, contributed to the non-convergence of the nonlinear estimator. While, standardizing these parameters is an option, we felt the gain in mathematical tractability did *not* off-set the loss in practical application of this procedure. Hence, we chose not to standardize but deal with the instability in our algorithms. In our tables, we delineate the number of cases where we did not have convergence of the nls estimator. This is an area that needs some attention in the nonlinear estimation arena.

In addition to this magnitude-effect, we also note that specific to our data and model fit, that the signs of the off-diagonal elements variance–covariance matrix Σ_i varied across locations. Referring to Table 1, we note that locations Omak (1), Chelan (2), Orondo (3), and Wenatchee (4) have similar signs in the off-diagonals than Naches (reference) and Wapato (6). These are all different from Royal City (5). This affected the calculation of our $H_{ii} = \Sigma_L + \Sigma_i$. This emphasizes the importance of reporting and reviewing the variance–covariance matrix for nonlinear models, and not just reporting and reviewing the variance (standard error) estimates.

In 1959, Richards notes [35, p. 299]: "Unfortunately, sound statistical methods cannot be suggested at present [. . .] for estimating the probability that any difference between [. . .] growth curves is statistically real". While we do not venture to believe we have solved this age-old problem, we feel our technique is a first step towards solving it. Specifically, we mathematically demonstrated that our method is asymptotically exact, controls well for family-wise Type I error, and has stable marginal power.

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Notes

1. Naches is a small, agrarian town where large quantities of tree-fruit are commercially grown: mostly apples, pears, cherries, and peaches. Naches is located in central Washington in the heart of Yakima valley, along the Naches River (a tributary of the Columbia River). Working with the Washington Tree Fruit Research, we chose Naches as the reference site during the design of the experiment. Based on this choice, appropriate resources were allocated to the Naches site enabling more frequent (Table 1) apple-growth observations.
2. Ratkowsky [34] defines the full Richards' model parameterization as a "particular unfortunate model because not only is its parameter-effects (PE) nonlinearity high but so too is its intrinsic (IN) nonlinearity" due to its overgenerality (p. 47). The three-parameter variation we chose is defined to be "one of the most versatile and useful models" as this symmetric model having good estimation properties of τ and a finite upper asymptote (see Section 5.3.2 on p. 128).
3. These estimates have desirable properties, in the sense that they are asymptotically unbiased, consistent with Ratkowsky [34]. Other methods will produce similar results, with some variations to the form of Σ_i. Specifically, we use the function `nls` within the statistical program `R` to fit the nonlinear model.

4. The code to compute the exact and simulated critical values using `R` requires the library `mvtnorm` and is available online `http://www.mshaffer.com/research/R-code/R-mvchi.txt`. For the code to apply this technique (including the `nls` model fitting), please contact Nairanjana Dasgupta.
5. Please refer to Table 1 for the sample sizes and design points. Only the sample sizes are needed to compute simulated critical values; for example, `N = c(483 571 575 595 691 667,1022)`; with an identification of how many parameters *k* in the model and which sample size is the referent *L*.
6. Note: we used the dependence matrix *R* to simulate our critical point.

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