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Statistical Evaluation of Data on Leaf Growth and Phyllochron as Derived from Leaf Length Measurements

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Abstract. Methods for testing equality of horizontal distances of subsequent parallel or common lines are suggested in this paper. Possibilities of using these methods are given on data concerning spring barley leaf growth.

Studying the biology of plants we have very often measured and described processes running in a given time interval with a constant speed and repeating themselves at time intervals of different lengths.

In our case we consider the dependence of the length of a growing leaf on the thermal time. In the given range the rate of growth is constant, therefore the dependence is of a linear type. In such a way individual leaves are growing over different time intervals. It is necessary to determine whether rates of growth of separate leaves are equal, i.e. whether the corresponding regression lines are parallel.

Further we are interested in comparison among phyllochrons that are determined by the differences of the thermal time between instants when two following leaves reach the given length.

Difficult and time-consuming measurements do not give corresponding results, often due to an insufficient or inadequate statistical evaluation. Whereas this way of collecting data is very frequent in the physiology of plants in this paper we propose a new method of statistical evaluation. Testing the parallelism of regression lines has already been used for a long time. However the further methods that we shall deal with, are not easily available although the basic ideas are simple.

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MATERIAL AND METHODS

A. Cultivation and measuring of plants

Spring barley (*Hordeum vulgare* L., cv. Korál) plants were cultivated in the nutrient solution Hoagland 1 (containing 1 mmol (NO_3^-) l^{-1} in solution), Hoagland 2 (5 mmol (NO_3^-) l^{-1}) and Hoagland 3 (15 mmol (NO_3^-) l^{-1}). Exact composition of nutrient solutions is given by Laštůvka and Minář (1967). The plants were cultivated in an unheated glasshouse.

The length of separate leaves was measured for 12 plants. The temperatures for each even hour were read from the thermograph records and the mean daily temperature was calculated. Values of instant temperatures less than 0 °C were added as zeros. Details of the cultivation and measurements were described by Nátr (1989).

B. Statistical evaluation

1. Notation

Let us introduce the necessary notation. We are dealing with m linear regression lines, i-th of them can be written as

$$y = \delta_i + \beta_i (x - \bar{x}) .$$

Parameter β_i characterizes the rate of growth of the i-th leaf. $\bar{x}_i$ indicates the mean of thermal times of this line, parameter δ_i indicates the length of the i-th leaf in the time x_i.

The hypothesis that all lines are parallel (all leaves have the same rate of growth) can be written as

$$H_\beta : \beta_1 = \beta_2 = \ldots = \beta_m (= \beta).$$

Let symbol y_0 denote some predetermined length of the leaf which we are interested in. The i-th leaf reaches this length in the time

$$x_{0i} = \bar{x}_i + (y_0 - \delta_i) / \beta_i .$$

It follows that i-th phyllochron equals the difference

$$\tau_i = x_{0,i+1} - x_{0i} .$$

The hypothesis that all phyllochrons are the same can be formulated as the requirement

$$H_\tau : \tau_1 = \tau_2 = \ldots = \tau_{m-1} (= \tau).$$

Now, we will append an important assumption, from which all suggested procedures follow. Deviations of measured values y (length of leaf) from that determined by linear dependency

$$y = \delta_i + \beta_i (x - \bar{x}_i)$$

are random, they have the same dispersion σ^2, they are stochastically independent and normally distributed.

2. Estimation of parameters of individual lines

For an estimation of the i-th regression line we have at our disposal the couples $(x_{i1}, y_{i1}), \ldots, (x_{in_i}, y_{in_i})$, where x_{ij} in our problem stands for thermal time and y_{ij} for the taken length of the i-th leaf. We have totally

$$n = \sum_{i=1}^{m} n_i$$

observations and suppose that $n > 2m$. For $i = 1, \ldots, m$ let us denote

$$\bar{x}_i = \left(\sum_{j=1}^{n_i} x_{ij} \right) / n_i ,$$

$$S_{xxi} = \sum_{j=1}^{n_i} (x_{ij} - \bar{x}_i)^2,$$

$$S_{xyi} = \sum_{j=1}^{n_i} (x_{ij} - \bar{x}_i)(y_{ij} - \bar{y}_i) ,$$

$$\bar{y}_i = \left(\sum_{j=1}^{n_i} y_{ij} \right) / n_i ,$$

$$S_{yyi} = \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_i)^2 ,$$

If it is true $S_{xxi} > 0$ for all i, then we can estimate the parameters of all m lines:

$$b_i = S_{xyi} / S_{xxi} , \qquad d_i = \bar{y}_i .$$

The residual sum of squares of the i-th line equals

$$RSS_{0i} = S_{yyi} - S^2_{xyi} / S_{xxi} .$$

Still we suppose that the lines are common, that they do not have to be parallel. The aggregate sum of squares equals

$$RSS_0 = \sum_{i=1}^{m} RSS_{0i} .$$

As the estimate of dispersion σ^2 we can use the statistic

$$s_0^2 = RSS_0 \,/\, (n - 2m) .$$

3. Testing parallelism of lines

For testing the hypothesis H_β it is useful to compute the estimate of the common slope β as $b = S_{xy} / S_{xx}$,

where

$$S_{xx} = \sum_{i=1}^{m} S_{xxi} ,$$

$$S_{xy} = \sum_{i=1}^{m} S_{xyi} ,$$

$$S_{yy} = \sum_{i=1}^{m} S_{yyi} .$$

If we suppose parallelism of lines, then the residual sum of squares equals

$$RSS_1 = S_{yy} - S_{xy}^2/S_{xx}$$

and estimate of dispersion σ^2 equals

$$s_1^2 = RSS_1/\,(n - m - 1) .$$

For testing the hypothesis of parallelism H_β (the growth rates of leaves are the same) we can use the statistic

$$F_\beta = ((RSS_1 - RSS_0) \,/\, (m - 1)) \,/\, (RSS_0/\,(n - 2m)) .$$

We will reject the hypothesis H_β on the level of significance α if

$$F_\beta \geqq F_{m-1,n-2m}(\alpha) ,$$

where the (upper) critical value of F distribution is on the right side.

In the case when we do not reject the hypothesis H_β, it interests us why we did so, which pair of lines are pairs of non-parallel lines (which pairs of leaves grow at a different rate).

The slopes of i-th and k-th lines (rate of growth of i-th and k-th leaf) are significantly different on the level of significance α by the Scheffe method (*e.g.* Anděl 1978) if

$$| b_i - b_k| \geqq s_0 ((m - 1) (1/S_{xxi} + 1/S_{xxk}) F_{m-1,n-2m}(\alpha))^{1/2}$$

In this case (estimates b_i and b_k and s^2 are independent) it is possible to apply the

Tukey method (Hayter 1984), too. Then, the slopes of i-th and k-th lines are significantly different when

$$|b_i - b_k| \geqq s_0\, q_{m,n-2m}(\alpha)\, ((1/S_{xxi} + 1/S_{xxk}) / 2)^{1/2},$$

where $q_{m,p}(\alpha)$ denotes the (upper) critical value of the studentized range. This second method gives as a rule a test with higher power (this test more frequently rejects the hypothesis when it is not true).

4. Testing equality of phyllochrons

Let us start with the estimate of the moment of attaining the given level y_0

$$\hat{x}_{0i} = \bar{x}_i + (y_0 - d_i) / b_i .$$

Evidently, this estimate is useful only in case of non-null b_i. In this case we estimate the i-th phyllochron as

$$\begin{aligned} t_i &= \hat{x}_{0,i+1} - \hat{x}_{0i} \\ &= (\bar{x}_{i+1} - \bar{x}_i) + (y_0 - d_{i+1}) / b_{i+1} - (y_0 - d_i) / b_i. \end{aligned}$$

For testing the hypothesis H_τ on equality of phyllochrons we want to know the variance of estimate $\hat{x}_{i,0}$. By means of routinely used approximation based on the Taylor expansion (*e.g.* Rao (1978), equation (6a.2.1)) we will get

$$\operatorname{var} \hat{x}_{0i} \cong \sigma^2 g_i ,$$

where we wrote

$$g_i = \frac{1}{n_i\, b_i^2} \left(1 + \frac{n_i\, (y_0 - \bar{y})^2}{b_i^2\, S_{xxi}}\right).$$

It follows that

$$\begin{aligned} &\operatorname{var} t_i \cong \sigma^2 (g_{i+1} + g_i), \\ &\operatorname{cov} (t_i, t_{i+1}) \cong - \sigma^2 g_{i+1} . \end{aligned}$$

Let us introduce a symmetric matrix **V** of order m–1 by

$$\begin{aligned} &v_{ii} = g_i + g_{i+1} , \\ &v_{i,i+1} = v_{i+1,i} = - g_{i+1} , \\ &v_{ij} = 0 \qquad \text{for } |i - j| > 1, \end{aligned}$$

vector $\mathbf{t}$ of elements $t_1, \ldots, t_{m-1}$ and vector of ones by

$$\mathbf{1} = (1,1, \ldots, 1)'.$$

For testing the hypothesis H_t we will use statistic

$$F_{\tau 0} = \frac{\mathbf{t}'\mathbf{V}^{-1}\mathbf{t} - (\mathbf{1}'\mathbf{V}^{-1}\mathbf{t})^2 / (\mathbf{1}'\mathbf{V}^{-1}\mathbf{1})}{(m-2)\, s_0^2}$$

The hypothesis is rejected on an approximate level of significance α, if

$$F_{\tau 0} \geqq F_{m-2,n-2m}(\alpha) .$$

The phyllochrons τ_i and τ_k are significantly different by the Scheffé method on an approximate level α, if

$$| t_i - t_k | \geqq s_0 \left((m-2)\, \mathbf{e}'_{ik}\, \mathbf{V}\, \mathbf{e}_{ik}\, F_{m-2,n-2m}(\alpha)\right)^{1/2} .$$

The symbol $\mathbf{e}_{ik}$ denotes a vector of zeros with the exception that the i-th element equals one and k-th element equals minus one.

5. Testing of equality of phyllochrons in case of parallel lines

In case of competent assumption that the lines are parallel (the leaves grow at the same rate), there are two possibilities for testing the hypothesis H_τ.

The approximate method, suggested in the previous chapter, can be adapted. To estimate the time of getting the given level y_0 we use the common estimate of slope, namely

$$x_{0i} = \bar{x}_i + (y_0 - d_i) / b .$$

Estimate of phyllochron then takes the form

$$\begin{aligned} t_i &= x_{0,i+1} - x_{0i} \\ &= (\bar{x}_{i+1} - \bar{x}_i) - (d_{i+1} - d_i) / b . \end{aligned}$$

Let us notice that in case of parallel lines (the same growth rate of the leaves) it makes no difference what value y_0 (what length of leaf) we used in definition of the phyllochron.

The approximate variance matrix $\sigma^2 \mathbf{V}$ of vector $\mathbf{t}$ is given by

$$v_{ii} = \frac{1}{b^2 n_i} + \frac{1}{b^2 n_{i+1}} + \frac{(d_{i+1} - d_i)^2}{b^4 S_{xx}} ,$$

$$v_{i,i+1} = v_{i+1,i} = -\frac{1}{b^2 n_{i+1}} + \frac{(d_{i+2} - d_{i+1})(d_{i+1} - d_i)}{b^4 S_{xx}},$$

$$v_{ij} = v_{ji} = \frac{(d_{j+1} - d_j)(d_{i+1} - d_i)}{b^4 S_{xx}} \qquad \text{for } |i - j| > 1.$$

For testing the hypothesis H_τ we use the statistic

$$F_{\tau 1} = \frac{\mathbf{t}'\mathbf{V}^{-1}\mathbf{t} - (\mathbf{1}'\mathbf{V}^{-1}\mathbf{t})^2/(\mathbf{1}'\mathbf{V}^{-1}\mathbf{1})}{(m-1)\, s_1^2}.$$

On approximate level α we will reject the hypothesis in the case when

$$F_{\tau 1} \geqq F_{m-2,n-m-1}(\alpha).$$

Approximately on the same level of significance phyllochrons τ_i and τ_k are different, when

$$|t_i - t_k| \geqq s_1 ((m-1)\, \mathbf{e}'_{ik}\, \mathbf{V}\, \mathbf{e}_{ik}\, F_{m-2,n-m-1}(\alpha))^{1/2}.$$

However, equality of phyllochrons can be tested in another way. According to hypothesis H_τ all values τ_i must be the same. If we suppose that all slopes are the same and non-null, it means that the quantities $\beta\tau_i = \tau_i^*$ are the same. In this case the hypothesis H_τ is equivalent to the hypothesis H_τ^* : $\tau_1^* = \tau_2^* = \ldots = \tau_{m-1}^*$ ($= \tau^*$). Parameter τ_i^* can be estimated by

$$t_i^* = b(x_{0,i+1} - x_{0i}) = bt_i$$
$$= b(\bar{x}_{i+1} - \bar{x}_i) - (d_{i+1} - d_i).$$

Exact variance matrix of vector $\mathbf{t}^*$ is equal to $\mathbf{V}^*$, where

$$v^*_{ii} = \frac{(\bar{x}_{i+1} - \bar{x}_i)^2}{S_{xx}} + \frac{1}{n_{i+1}} + \frac{1}{n_i},$$

$$v^*_{i,i+1} = v^*_{i+1,i} = \frac{(\bar{x}_{i+2} - \bar{x}_{i+1})(\bar{x}_{i+1} - \bar{x}_i)}{S_{xx}} - \frac{1}{n_{i+1}},$$

$$v^*_{ij} = \frac{(\bar{x}_{j+1} - \bar{x}_j)(\bar{x}_{i+1} - \bar{x}_i)}{S_{xx}} \qquad \text{for } |i - j| > 1.$$

As test criterion we will use

$$F_{\tau^*} = \frac{\mathbf{t}^{*\prime}\mathbf{V}^{*-1}\mathbf{t}^* - (\mathbf{1}'\mathbf{V}^{*-1}\mathbf{t}^*)^2/(\mathbf{1}'\mathbf{V}^{*-1}\mathbf{1})}{(m-1)\, s_1^2}$$

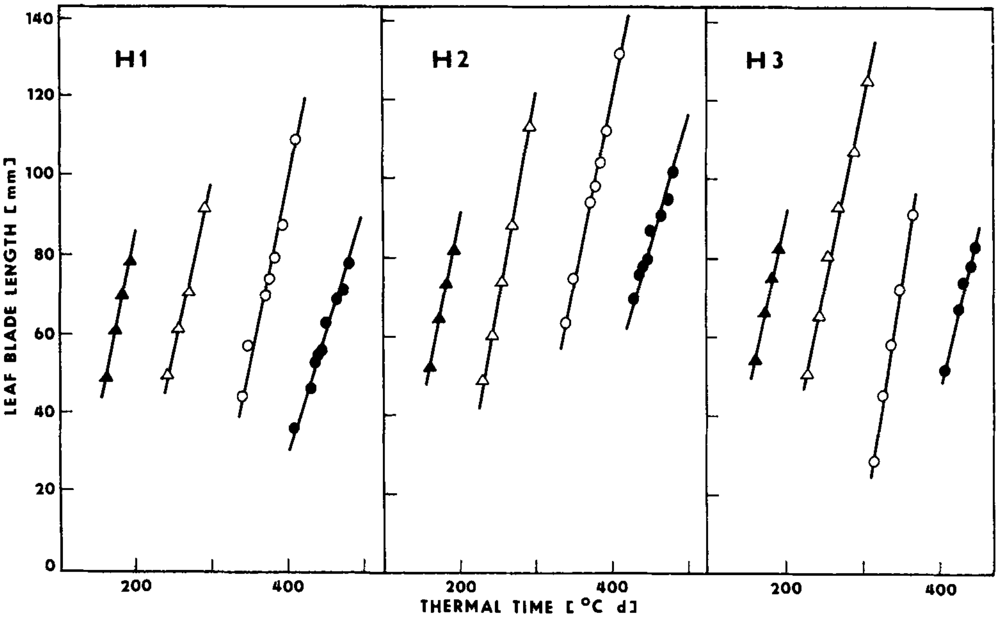

Figure 1. The dependence of the leaf blade length of the 1st (▲), 2nd (△), 3rd (○), 4th (●) leaf of spring barley plant on sum of daily mean temperature greater than 0 °C (°C D). Plants were cultivated in Hoagland 1 nutrient solution (H1), Hoagland 2 (H2), Hoagland 3 (H3).

Now, we will reject the hypothesis on the exact level of significance α, if it true that

$$F_{\tau^*} \geqq F_{m-2,n-m-1}(\alpha) .$$

Quantities τ^*_i and τ^*_k (so phyllochrons τ_i and τ_k) are significantly different by the Scheffé method if

$$|t^*_i - t^*_k| \geqq s_l((m-2)\, e'_{ik} V^* e_{ik} F_{m-2,n-m-1}(\alpha))^{1/2}.$$

RESULTS AND COMMENTS

There are data on four leaves for each of three nutrient solutions (Fig. 1.). The parameter estimates of common lines are stated in Table 1. For none of the solutions can the lines be supposed parallel as can be seen from Table 2. In all cases it is due to the rate of growth of the 4-th leaf, which does not constitute a homogenous group with the others. Moreover, for the solutions Hoagland 1 and Hoagland 2 it differs from the others on the 5 % level (Table 3).

Time x_{0i} of attaining the given length y_0 depends on the choice of this value. When we select $y_0 = 50$ mm, we will get the estimates given in Table 4. The table gives estimates of time taken to attain leaf length 50 mm computed under the assumption

TABLE 1

Parameter estimates of regression lines of dependency of leaf length on thermal time. For description see text.

	Hoagland 1				Hoagland 2				Hoagland 3			
i	n_i	d_i	b_i	$\hat{\sigma}_i^2$	n_i	d_i	b_i	$\hat{\sigma}_i^2$	n_i	d_i	b_i	$\hat{\sigma}_i^2$
1	4	64.50	0.9145	1.355	4	67.50	0.9145	1.355	4	69.25	0.8860	2.410
2	4	67.75	0.8753	2.990	5	75.80	1.0593	3.804	6	86.17	0.9102	8.579
3	7	74.29	0.8411	12.817	7	96.00	0.9207	2.998	5	58.80	1.1867	13.328
4	9	58.56	0.5792	3.848	8	83.88	0.5642	3.842	6	71.50	0.7624	2.860

TABLE 2

Tests of parallelism of regression lines, which estimates are given in Table 1.

	F_β	Significance level	degrees of freedom	
Hoagland 1	9.641	0.0007	3	16
Hoagland 2	32.606	0.0000	3	16
Hoagland 3	6.526	0.0063	3	13

TABLE 3

Homogeneous groups of slopes from Table 1 by means of Scheffé and Tukey multiple comparison method (every column represents one group, a star indicates incidence in this group)

	Hoagland 1		Hoagland 2		Hoagland 3	
i	Scheffé	Tukey	Scheffé	Tukey	Scheffé	Tukey
1	**	*	*	*	**	**
2	*	*	*	*	**	**
3	*	*	*	*	*	*
4	*	*	*	*	*	*

TABLE 4

Estimates of thermal time for taking leaf length equal 50 mm in models with individual and with common slopes (parallel regression lines). In parentheses are given standard errors of estimates.

	Hoagland 1 slope		Hoagland 2 slope		Hoagland 3 slope	
i	individual	common	individual	common	individual	common
1	159.4(2.3)	155.6(2.8)	156.1(1.9)	154.8(2.8)	153.5(3.1)	155.0(2.2)
2	241.0(2.2)	238.2(2.9)	230.8(1.2)	225.0(2.8)	223.9(2.1)	225.6(2.4)
3	343.7(1.8)	339.7(2.6)	322.6(1.8)	318.8(3.5)	331.8(1.1)	329.9(1.8)
4	430.5(1.8)	433.6(1.8)	389.8(4.0)	410.3(2.8)	404.3(3.4)	409.8(1.9)

TABLE 5

Estimates of phyllochrons (°C d) of common lines (see Table 4) and theirs incidence to groups by means of Scheffé multiple comparison method.

	Hoagland 1		Hoagland 2		Hoagland 3	
i	$\hat{\tau}_i$		$\hat{\tau}_i$		$\hat{\tau}_i$	
1	82.6	*	74.7	*	70.4	*
2	101.7	*	91.8	*	107.9	*
3	86.8	*	67.2	*	72.5	*

TABLE 6

Tests of equality of phyllochrons (see **TABLE 4**).

	F_τ	Significance level	Degres of freedom		Estimate of common value
Hoagland 1	7.261	0.0057	2	16	91.6
Hoagland 2	14.857	0.0002	2	16	81.8
Hoagland 3	37.681	0.0000	2	13	91.1

that the lines are parallel, too. As we found, this assumption is not satisfied in our data. It is interesting to notice, that those two estimates do not differ too much (differences are smaller then twice the standard error as a rule). For a different value of y_0 the result can be dissilimar.

The estimates of phyllochrons derived from the estimates of getting a given value for $y_0 = 50$ mm are given in Table 5. It is not possible to consider them as equal in any case, because the relevant F statistics are highly significant (Table 6). From Table 5 it is evident that this is due to the high value of the second phyllochron, therefore by the greater distance between times of getting the given value $y_0 = 50$ mm for the second and third leaf.

This method of statistical evaluation was used in experiments by Tesařová and Nátr (1990), where the importance of thermal time for the description of the rate of leaf growth and values of phyllochron is discussed. Experimental data given in our paper are used to demonstrate the method of statistical evaluation. These results are part of a bigger series of experiments, which will be published in a special paper.

The program used written in Turbo Pascal 5.0 for IBM XT/AT computer can be obtained from the first author.

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