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FITTING LOGISTIC-TYPE CURVES TO EXTENSION GROWTH DATA FOR LEAVES OF GRASS SPECIES BY MEANS OF THE MAXIMUM LIKELIHOOD PROGRAM: ANALYSIS OF LEAF EXTENSION IN *LOLIUM TEMULENTUM* AT OPTIMAL AND CHILLING TEMPERATURES

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THOMAS H. and POTTER J. F. *Fitting logistic-type curves to extension growth data for leaves of grass species by means of the Maximum Likelihood Program: analysis of leaf extension in* Lolium temulentum *at optimal and chilling temperatures.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **25,** 157–163, 1985.—Grasses in temperate regions are exposed to suboptimal temperatures for part of their growth cycles. The responses of leaf extension to chilling temperatures in *Lolium temulentum* were quantified by fitting leaf lengths to generalized logistic (Richards) functions by means of the Maximum Likelihood Program. Primary and derived Richards function parameters exhibiting clear sensitivity to low temperature included: asymptotic maximal length, which was reduced by 60% at 5°C as compared with 20°C; duration of leaf growth, which was increased 2- to 6-fold at the chilling temperature; and absolute and relative extension rates, which were markedly depressed at 5°C. Plastochron indices and mean rates of leaf production were derived from 6-leaf *Lolium* plants at 20°C and 5-leaf plants at 5°C, based on leaf lengths estimated from fitted functions. Chilling sensitivities, measured as temperature coefficient Q_{10}, were derived for each of the growth rate parameters. The rate of leaf appearance was the least sensitive, with a Q_{10} of close to 2. Absolute and relative extension rates gave consistently higher Q_{10} values, with the latter exhibiting an apparent adaptive trend towards lower sensitivity with prolonged exposure to 5°C. These results are discussed in relation to the question of the relative contributions to the behavior of grasses at chilling temperatures of direct passive reactions and positive acclimation responses.

INTRODUCTION

DURING the life-cycles of perennial or overwintering annual plant species from temperate regions there may be one or more, often prolonged, periods when ambient temperatures are considerably below the optimum for growth. Forage grasses and autumn-sown cereals are examples of important crops which do not possess a true winter dormancy mechanism and which show significant, if reduced, growth in the cold season. Within grass species there exists considerable inter-varietal and ecotypic variation for the ability to grow at chilling temperatures (that is, low positive temperatures in the range 0–10°C)(15): it is of interest to be able to quantify these growth responses, particularly when investigating the cellular basis of temperature perception and its transduction into increase in mass and dimensions. POLLOCK *et al.*(13) approached this problem with conventional growth analysis techniques. By comparing Q_{10} values for relative growth rate, determined by destructive dry mass determinations, and for photosynthetic capacity they showed that the growth of *Lolium temulentum* at chilling temperatures is not limited by the supply of fixed carbon and that the excess of carbon assimilate supply over that demanded by the growth processes is diverted into fructan reserves.

An alternative, non-destructive, approach is that of THOMAS(17) in which measurements of leaf lengths between the time of emergence, or of transfer to low temperature, and the attainment of full expansion are fitted to the Richards function.(3,4,18) Growth rates and other derived parameters were compared for *Lolium* plants developing at 20°C and exposed to 5 or 2°C at emergence, mid-expansion or maturity of the fourth leaf. The results corresponded closely with those obtained by the destructive dry mass procedure.(19) The curve-fitting approach offers a convenient way of reducing a mass of detailed, easily obtained data into a few biologically meaningful growth parameters, complete with estimates of statistical significance. The original analyses were performed using dedicated programs for the fitting of Richards and Gompertz functions.(2,18) We were interested in developing a standard 'production-line' procedure for curve-fit analyses using a widely available computer modelling package. We describe here the use of the Maximum Likelihood Program (MLP)(16) to fit various forms of the Richards function, and show how the primary and derived growth parameters are used to characterize the production and growth of *L. temulentum* leaves over a prolonged period at chilling temperatures.

MATERIALS AND METHODS

Plant material

Seeds of *L. temulentum* L. (variety Ba 3081 summer annual) were germinated in a controlled environment chamber at 20°C on moist filter paper. After 7 days selected uniform seedlings were transferred to a nutrient growing medium as described by POLLOCK.(11) One half of the plants were kept at 20°C and the remaining plants were assigned to a 5°C growth chamber. The light regime was an 8 hr photoperiod at 40 W/m^2, at both temperatures. Lengths of primary leaves were measured from the point of root insertion to the tip. Lengths of subsequent leaves were measured from the point of emergence from the enclosing sheath of the previous leaf.

Table 1. Summary of parameter symbols employed by Maximum Likelihood Program and their identification with terminology adopted by THOMAS(18)

Parameter	Generalized logistic $Y = A + C/\{1 + T \exp[-B(X-M)]\}^{(1/T)}$	Richards function $L = A[1 \pm \exp(b-kt)]^{-1/n}$	Derivation*
Leaf length (mm)	Y	L	
Time (days)	X	t	
Inflexion point (days)	M	m	$(b-\log_e n)/k$†
Rate constant	B	k	
Exponent	T	n	
Upper asymptote (mm)	C	A	
Lower asymptote (set to zero)	A	–	
Mean relative extension rate (per day)	C11	$\bar{R}$	$k/(n+1)$‡
Mean absolute extension rate (mm/day)	C12	$\bar{G}$	$kA/(2n+4)$
Slope parameter	C13	b	$km+\log_e n$
Duration of growth (days)	C14	D	$(2n+4)/k$

* For details of derivations of secondary parameters see CAUSTON *et al.*,(3) CAUSTON and VENUS(4) and THOMAS.(18)

† In the limiting case as $n \rightarrow 0$ (Gompertz function) $m = b/k$.

‡ In the Gompertz function $\bar{R} = k$.

Curve-fitting

Extension growth data were fitted to Richards functions using the Maximum Likelihood Program running on a DEC VAX 11/750. MLP offers the Generalized Logistic (GLOG)(10) as a standard model. This differs from the Richards function in some details of parameterization and also in fitting a low asymptote as well as an asymptotic maximal value. By fixing the low asymptote to zero using the MLP option CAS = 0 the GLOG model becomes equivalent to the Richards function. Table 1 presents a comparison of primary and derived parameter symbols associated with GLOG and with the Richards function as defined by THOMAS.(18) For consistency this paper employs the latter terminology throughout, except where examples of MLP code are presented. The data were replicated, in the sense that different plants were measured. Ideally the standard errors for parameters and functions of parameters should be based on the within-t variance. This is the unbiased estimate of error, or Pure Error, which is used also to estimate goodness of fit. MLP allows the use of Pure Error with the option CVA = REP.

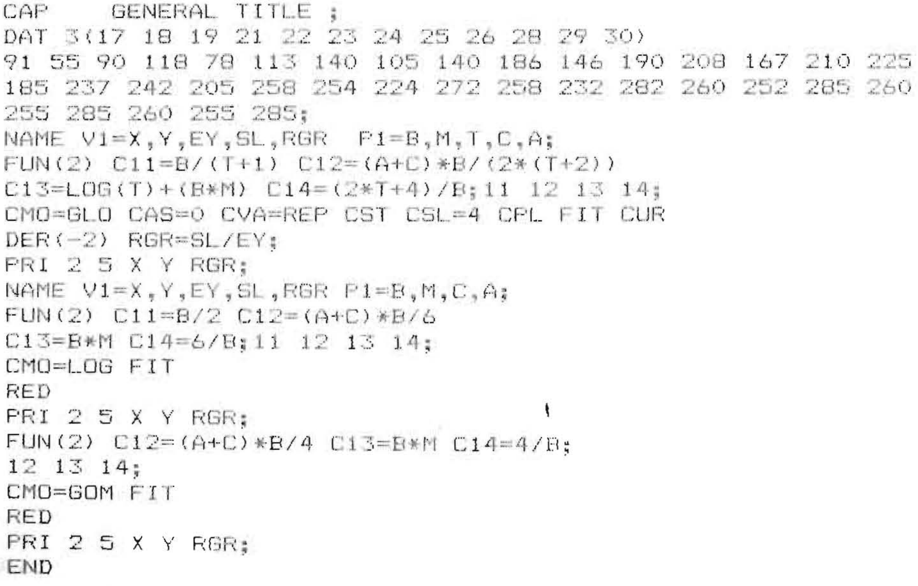
Data must be presented to the model in groups of L values for each t. That is, the L values are premultiplied by the replication. The following functions are defined: $\bar{R}$ = weighted mean relative extension rate (units = t^{-1}), $\bar{G}$ = weighted mean absolute extension rate (units = Lt^{-1}), b (see Table 1) and D = duration of growth (units = t). For a discussion of these functions see THOMAS.(18)

Options are set for fitting GLOG and for two special cases: Logistic (LOG, where $n = 1$) and Gompertz (GOMP, for n approaching 0). GLOG often reduces to LOG or GOMP so these are fitted routinely first time through to avoid running again. In each case instantaneous relative extension rate (R) is calculated as slope/fitted L value. Figure 1 presents a typical MLP program to fit these growth curves.

Plastochron index and Q_{10}

Plastochron indices were calculated from fitted estimates of leaf length L according to the following equation.(9,18)

$$P_t = p + [(\log_e EL_p - \log_e 10)/(\log_e EL_p - \log_e EL_{p+1})], \quad (1)$$



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CAP     GENERAL TITLE ;
DAT 3(17 18 19 21 22 23 24 25 26 28 29 30)
91 55 90 118 78 113 140 105 140 186 146 190 208 167 210 225
185 237 242 205 258 254 224 272 258 232 282 260 252 285 260
255 285 260 255 285;
NAME V1=X,Y,EY,SL,RGR  P1=B,M,T,C,A;
FUN(2) C11=B/(T+1) C12=(A+C)*B/(2*(T+2))
C13=LOG(T)+(B*M) C14=(2*T+4)/B;11 12 13 14;
CMO=GLO CAS=0 CVA=REP CST CSL=4 CPL FIT CUR
DER(-2) RGR=SL/EY;
PRI 2 5 X Y RGR;
NAME V1=X,Y,EY,SL,RGR P1=B,M,C,A;
FUN(2) C11=B/2 C12=(A+C)*B/6
C13=B*M C14=6/B;11 12 13 14;
CMO=LOG FIT
RED
PRI 2 5 X Y RGR;
FUN(2) C12=(A+C)*B/4 C13=B*M C14=4/B;
12 13 14;
CMO=GOM FIT
RED
PRI 2 5 X Y RGR;
END
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FIG. 1. A program for fitting leaf growth data to Generalized Logistic, Logistic and Gompertz functions in MLP.

where P_t is the plastochron index at time t, p is the ordinal of the smallest leaf longer than 10 mm at time t, EL_p is the estimated L of leaf p at this time and EL_{p+1} the estimated L of leaf $(p+1)$. A program written in BASIC and running on a CBM 4032 microcomputer derived daily plastochron indices from Richards function parameters entered for leaves 1–N. The program produces a table and graph of P_t and t and also performs a linear regression of P_t on t. The regression slope gives the mean rate of leaf appearance and its reciprocal the mean duration of the plastochron.$^{(18)}$ Q_{10}s over the 5–20°C range were calculated by the standard equation

$$Q_{10} = (r_{20}/r_5)^{0.667}, \tag{2}$$

where r_{20} and r_5 are the rates of a given process at 20 and 5°C, respectively.

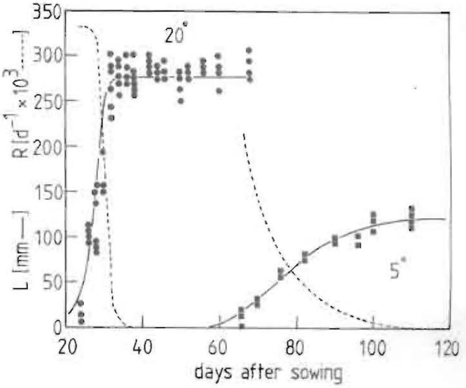

FIG. 2. Richards function curves and derived relative extension rates fitted to lengths of *L. temulentum* fourth leaves growing at 20 and 5°C under an 8 hr photoperiod.

RESULTS AND DISCUSSION

Richards functions were fitted to measurements of length taken over the period between emergence and full expansion for the first six leaves of *L. temulentum* plants grown at 20°C and leaves 1–5 of plants grown at 5°C. The experiment was terminated 110 days after sowing and provided incomplete data for leaf 6 at 5°C, which was therefore omitted from the analysis. Figure 2, by way of example, compares growth of leaf 4 at 20 and 5°C in terms of original length measurements, fitted Richards functions and derived relative extension rate curves. Table 2 presents a summary of the parameters defining extension and its response to temperature for all leaves. Standard errors are omitted for the sake of clarity. The differences between the 20 and 5°C treatments are highly significant ($P < 0.001$) for parameters A, m, D, $\bar{R}$ and $\bar{G}$ in all cases.

Estimated length at full expansion (A) increased with each successive leaf at both temperatures. Leaves growing at 5°C achieved an average of about 40% of the maximal size at 20°C, but

Table 2. Primary and derived Richards function parameters for leaves of Lolium temulentum *growing at optimal and chilling temperatures under an* 8 hr *photoperiod*

Leaf	Temperature (°C)	Parameter A	k	m	n	b	D	$\bar{R}$	$Q_{10}^{\bar{R}}$	$\bar{G}$	$Q_{10}^{\bar{G}}$
1	20	113	0.83	9.09	1.0	7.23	7.55	0.42	3.97	15.7	4.72
	5	67	0.32	19.2	5.08	7.79	44.1	0.053		1.53	
2	20	166	0.61	14.3	0	6.58	8.69	0.61	2.55	25.3	4.39
	5	58	0.26	37.9	0.74	9.46	21.3	0.15		2.75	
3	20	211	1.01	20.8	1.0	21.0	5.94	0.51	2.49	35.5	5.20
	5	70	0.26	56.2	1.0	14.4	23.5	0.13		2.99	
4	20	274	1.23	28.9	3.73	36.9	9.31	0.26	1.92	29.4	4.72
	5	117	0.10	73.8	0	7.23	40.8	0.098		2.87	
5	20	335	0.24	32.5	0	7.85	16.5	0.24	1.83	20.2	2.79
	5	134	0.19	94.9	1.0	18.4	30.9	0.097		4.33	
6	20	347	0.26	39.4	0	10.2	15.5	0.26		22.4	

were not notably different in width, although they characteristically remained tightly rolled for a prolonged period after emergence. The difference in final length between 20 and 5°C grown leaves is reflected in leaf area and fresh weight and also in the number of mesophyll cells per leaf,(14) suggesting that cell division is particularly sensitive to chilling temperatures. It is not certain to what extent these reactions are direct, reversible thermodynamic responses to decreased temperature or true adaptive modifications. Leaves of *Lolium* plants grown at 20°C and transferred to 5°C at mid-expansion also attain a significantly smaller final length than leaves remaining at the higher temperature.(17) Moreover, reductions in growth rate on exposure to low temperatures are extremely rapid—we have observed the establishment of a stable new rate within a few minutes of transfer from 20 to 5°C and an equally rapid reversal on return to higher temperatures (ref. 19 and unpublished observations). Shifts in the relative distribution of assimilated carbon between growth and reserve carbohydrate accumulation also occur over a similarly brief time-span when *Lolium* plants are exposed to chilling temperatures.(12) It could be argued that these represent freely reversible perturbations rather than responses of an adaptive nature.

Pollock *et al.*,(13) however, observed an enhancement of photosynthetic capacity, particularly when measured at the chilling temperature, in existing leaves of *Lolium* on transfer from 20 to 5 or 2°C. Leaves initiated and developing entirely at 5°C exhibited no such acclimation of photosynthetic capacity at maturity. Indeed, on a cell and chloroplast basis, photosynthetic capacity was significantly lower at 5°C than at 20°C; but whereas capacity declined steadily from ligule formation at 20°C, at 5°C photosynthesis actually increased to a new maximum in the same period.(14) Inhibition of leaf unrolling is also of interest in the context of acclimation responses since unrolling is believed to be under phytochrome control.(1) High-resolution growth studies have revealed a complex relationship between diurnal rhythm, photoperiod and temperature.(19) Photomorphogenetic reactions certainly appear to be particularly sensitive to temperature and may be important in triggering longer-term acclimation processes.

Of the primary parameters other than A, only k and m were obviously relatable to leaf number or temperature treatment or both (Table 2). The significance of m will be discussed below in relation to the plastochron index. The biological meaning of k is most evident in the parameters derived from it, namely D, $\bar{G}$ and $\bar{R}$ (Table 1). Increased D and decreased $\bar{G}$ at 5°C indicate inhibition not only of the production of leaf cells by division but also of the rate at which the cells expand. Cell expansion is a function of the availability of water and the extensibility properties of the cell walls. Chilling is known to cause large perturbations in the water status of expanding grass leaf tissue by decreasing water uptake through the roots and altering the water-relations of the lamina.(8,20) The intrusion of water-stress as an additional factor limiting growth at low temperatures may be accompanied by Q_{10} values higher (sometimes enormously so)(19) than those for simple temperature-sensitive metabolic processes.(7) Q_{10} figures in excess of 4.0 were apparent for $\bar{G}$ of the first four leaves of *Lolium* between 5 and 20°C (Table 2). Between 5 and 2°C Q_{10}s of over 20 and as high as 62 have been observed.(19) The Q_{10} for leaf 5 $\bar{G}$ was appreciably lower than for the preceding four leaves. We are not able to say whether this represents the appearance of an adapative trend in leaves which, unlike leaves 1–3, are not present as initials in the embryo prior to seed germination.(5) An apparent trend in temperature acclimation is quite strongly suggested by the Q_{10} values of $\bar{R}$, however (Table 2): it seems that the return, in terms of leaf extension, on the investment in leaf tissue at the lower temperature improves progressively with continued exposure to chilling conditions.

One of the objectives of this study was to establish plastochron indices for *L. temulentum*, allowing physiological and metabolic changes occurring at 20 and 5°C to be expressed on a developmental time-scale.(9) Figure 3 presents plastochron indices for 20 and 5°C grown *Lolium* derived from estimated lengths (EL) given by fitted Richards functions. Ontogenetic trends in photosynthetic capacity at optimal and chilling temperatures expressed on the basis of these indices are described by Pollock *et al.*(14) Linear regression of plastochron index on d after sowing gave a highly significant correlation coefficient in

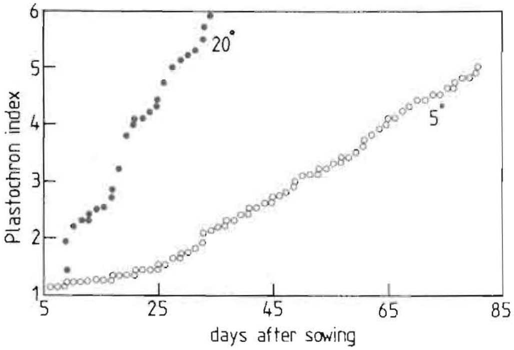

FIG. 3. Plastochron indices calculated from estimated lengths given by Richards functions fitted to measurements of *L. temulentum* leaves growing at 20 and 5°C under an 8 hr photoperiod.

each case and provided an estimate of the mean duration of the plastochron and its reciprocal, the mean rate of leaf appearance. These data are given in Table 3, where they are compared with values obtained from linear regression of parameter *m* on leaf number. The inflexion point of the fitted growth curve, *m*, exhibited a pronounced trend with successive leaves (Table 2). The slope of the regression of *m* on leaf ordinal represents the mean number of *d* between approximately mid-expansion of successive leaves and may be taken as another measure of plastochron duration. Plastochron intervals and rates of leaf appearance calculated in this way compare very closely with those obtained from *EL*s (Table 3). Chilling extended the time between the appearance of successive leaves by a factor of 3 as compared with 20°C. A Q_{10} of about 2 for the rate of leaf appearance indicates that the production of leaf initials at the apex is less sensitive to chilling inhibition than are their subsequent growth and development.

It has been argued that fitting empirical models to experimental observations does no more than re-describe the data and is incapable of providing insights into biological mechanism. HUNT(6) has summarized the case in favor of the so-called 'functional' approach, emphasizing the economy of means by which primary data may be obtained and by which large numbers of observations, often dauntingly complex *en masse*, can be reduced to a few readily comprehensible parameters. This in turn allows comparisons to be made of data from different origins, such as those arising from the kind of temperature treatment described here. Areas for further investigation are readily identified in this way. Thus we perceive that the processes of cell division and cell expansion invite closer study if the mechanism of modified growth of *Lolium* and other temperate grasses at chilling temperatures is to be understood.(13,15,19) Investigations into the temperature sensitivities of primary growth processes in the intercalary meristem region are currently in progress.

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Table 3. Estimates of plastochron duration and rate of leaf appearance for Lolium temulentum *plants grown at 20 and 5°C*

	Temperature (°C)	Derived from *m*	Derived from *EL*
Correlation coefficient	20	0.914	0.818
	5	0.999	0.997
Mean duration of plastochron (days) ± S.E.	20	6.12 ± 0.24	6.52 ± 0.72
	5	18.73 ± 0.32	19.00 ± 0.90
Mean rate of leaf appearance (leaf/day) ± S.E.	20	0.163 ± 0.006	0.153 ± 0.019
	5	0.053 ± 0.001	0.053 ± 0.003

The table compares values derived by linear regression of inflexion point *m* on leaf number with those obtained by the method of THOMAS,(18) which uses leaf lengths estimated from the fitted curves (*EL*). The correlation coefficients are all significant at $P < 0.001$.

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