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Temperature sensitivities of *Festuca arundinacea* Schreb. and *Dactylis glomerata* L. ecotypes

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SUMMARY

The thermal responses of leaf growth and senescence were measured in four populations of *Festuca arundinacea* Schreb. and three of *Dactylis glomerata* L. with a range of geographical origins. Leaf extension was quantified by fitting logistic curves to lengths measured for up to 120 d in controlled environments at 20 °C or after transfer to 5 °C at emergence of the fourth leaf. Electronic position transducers were employed to observe rapid adjustments in leaf elongation rate in response to changes in the temperature experienced by the growing zone. Leaf segments were incubated in darkness on a thermal gradient bar giving a range of temperatures between 0 and 20 °C, and chlorophyll and protein were determined after 4 d of senescence. For each process, temperature sensitivities were determined as Q_{10}, to give a profile of thermal response for each ecotype. Correlations between processes across ecotypes enabled possible relationships between temperature perception mechanisms to be identified. In their responses to chilling temperatures, mean extension rates measured over a period of one or more plastochrons were not closely related to short-term rates. There was a significant negative correlation between chlorophyll stability in excised leaf tissue and mean long-term extension rates. In general, ecotypes from the coldest habitats were the most sensitive to low temperature inhibition.

Key words: Thermal response, leaf growth and senescence, Q_{10}, *Dactylis glomerata*, *Festuca arundinacea*.

INTRODUCTION

Suboptimal temperatures constrain all aspects of leaf development in the temperate grasses. The rate of leaf appearance declines during the winter, as does foliar senescence, so that the mean number of live leaves per axis generally remains relatively unchanged over the annual cycle (Robson, Ryle & Woledge, 1988). Creber, Davies & Francis (1993) found that although the duration of the cell cycle in the roots of *Dactylis glomerata* ecotypes was extended at low temperature, the proportion of cells in the meristem engaged in making new cells increased. Cell extension is extremely responsive to temperature, over the time-scale of seasons and also down to the limits of resolution of electronic growth-transducers (Stoddart *et al.*, 1986; Pollock & Eagles, 1988). Under winter conditions, as a result of the differential sensitivities of cell production and extension, juvenile leaf tissue might accumulate in an unexpanded form within the sheaths of older leaves so that when the temperature constraint is relieved there is an explosion of growth (Pollock & Eagles, 1988).

Grasses of the *Festuca–Lolium* complex and the genus *Dactylis* are not only economically important forages but also fruitful subjects for physiological studies of thermal adaptation (Sugiyama, 1986; Humphreys, 1987; Tcacenco, Eagles & Tyler, 1989; Wilkins, 1991; Fujimoto, 1993). Earlier studies on *Festuca arundinacea* Schreb. and *Dactylis glomerata* L. described wide variations between natural populations and derived cultivars in the extent to which different components of leaf growth and survival respond to temperature limitations (Chatterjee, 1961; Robson, 1967; Eagles & Østgård, 1971; Negri *et al.*, 1984). More recently, methods have been developed for looking in detail, and with higher resolution, at grass leaf growth and development and the constituent temperature-sensitive processes. Thomas (1983) and Thomas & Potter (1985) investigated the temperature responses of leaf extension in *Lolium temulentum* under controlled environment conditions. Growth was analysed by fitting logistic-type curves, and the primary and derived parameters were used to quantify the extent to which the

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component processes of leaf initiation and expansion are sensitive to suboptimal temperatures. These processes were subsequently examined with increased resolution in time and space by means of small-scale growth analysis procedures, growth transducers, single-cell pressure probe methods and biochemical measurements (Pollock *et al.*, 1983; Thomas & Stoddart, 1984; Stoddart *et al.*, 1986; Schnyder, Nelson & Coutts, 1987; Ougham, 1987; Thomas *et al.*, 1989). In a study of the contrasting reaction of spring and winter oats to chilling temperatures, Thomas, Stoddart & Potter (1980) found characteristic varietal differences in the thermal behaviour of a number of physiological processes, including the senescence of excised leaves. A progressive approach to the analysis of growth and development, closing in on smaller and smaller time intervals and volumes of tissue, helps define, in cellular and molecular terms, critical points in plant responses to low temperatures (Pollock & Eagles, 1988; Howarth & Ougham, 1993). It has potential applications in understanding growth/temperature response mutants and genetic variants, including ecotypes; recent examples of this approach with graminaceous species include studies on the *slow-to-green* mutant of *Lolium temulentum* (Ougham *et al.*, 1992) and the *slender* mutant of barley (Schünmann, Ougham & Turk, 1994).

In the present paper we employ curve-fitting and growth-transducer methods to measure thermal responses of leaf extension at different levels of time-resolution in four *Festuca arundinacea* and three *Dactylis glomerata* populations with a range of geographical origins. The temperature sensitivity of senescence in isolated leaf tissue was also examined. We were particularly interested in assessing the extent to which low temperature might influence different aspects of growth and senescence via common perception and transduction mechanisms.

MATERIALS AND METHODS

Plant material

Seeds of grass ecotypes and cultivars were obtained from the germplasm collection at IGER Aberystwyth and represent material obtained from various sources by the Institute Plant Introduction Unit (Tyler & Chorlton, 1978; Tyler, Chorlton & Thomas, 1987). Data are presented here for three natural populations each of *Festuca arundinacea* (*Fa*) and *Dactylis glomerata* (*Dg*) as described in Table 1. The *Fa* variety S170 was also included. As an estimate of the length of the growing season in each of the habitats from which the lines were collected, Table 1 gives the number of days per year with a mean air temperature of 6 °C or greater (Smith, 1984). We refer to the various lines as ecotypes or populations. Plants were grown from seed in John Innes No. 3 compost in a controlled environment at 20 °C, 8 h light/16 h dark photoperiod, 350 μmol m^{-2} s^{-1} PAR light flux. At the time of emergence of leaf 4, some plants were transferred to 5 °C (identical light regime) and lengths of leaves 4 and 5 (*Fa*) or 4 only (*Dg*) in five replicate plants were measured. In the subsequent discussion, *growth* refers to *linear extension*, except where relative growth rates based on dry weight measurements are presented.

Kinetic measurement of leaf extension

High-resolution measurements of growth responses to temperature were made using the instrumentation described by Stoddart *et al.* (1986). Plants were grown at 20 °C until the fourth leaf was at approximately half full expansion. The shoot base was enclosed in a small jacket supplied with coolant from a temperature-controlled circulation unit. Temperatures in the growth studies reported here are those of the plant tissue, monitored with a small thermocouple probe within the jacket. The tip of the growing leaf was connected to a linear voltage displacement transformer (LVDT) and a counterweight. After extension had stabilized at ambient temperature ($\sim$ 25 °C), cooling commenced at 1 °C min^{-1}. The leaf base was held at $-0{\cdot}5° \pm 0{\cdot}3$ °C for 20 min and then rewarmed at 1 °C min^{-1}. The respective temperatures at which measurable extension ceases during cooling and resumes on warming are useful indices of the physiological state of the growing tissue and have been shown to be changed by factors such as long-term thermal acclimation and applied plant growth regulators (Stoddart *et al.*, 1986; Stoddart & Lloyd, 1986). The growth rate at a given temperature is frequently higher on the re-warming side of the cycle than at the same temperature during cooling. This hysteresis is a measure of 'stored growth', the unexpressed potential for extension accumulated at suboptimal temperature, and is quantified as H, the area enclosed by the cooling and warming curves between the lowest and highest temperatures recorded in the experiment (Stoddart *et al.*, 1986). Linear regression of growth rate on temperature calculated separately for the cooling and warming parts of the cycle gave correlation coefficients of better than 0·92 for all genotypes. Stop and re-start temperatures were given by regression intercepts and Q_{10} for cooling and re-warming was calculated from regression slopes.

Leaf extension in two of the *Dg* lines was also measured under controlled photoperiod conditions using the optoauxanometer device described by Thomas & Stoddart (1984) and Thomas, Rowland & Stoddart (1984).

Leaf senescence

Sections 1 cm long were taken from the central portions of youngest fully-expanded leaves of 20 °C-grown plants 38 d after sowing. Segments from the

Table 1. *Origins of* Festuca arundinacea *and* Dactylis glomerata *ecotypes used in the present study*[a]

Ecotype	Location	Latitude	Longitude	Altitude (m above sea level)	Mean T°C: Coldest month	Mean T°C: Warmest month	Days per year with mean T°C ⩾ 6 °C
Festuca arundinacea							
S170[b]					4–5	15–17	220–250
Bn379	Near Thala, Tunisia	35° 35′ N	8° 40′ E	1150	5·8	25·7	350
Bn467	Ifrane, Morocco	33° 31′ N	5° 10′ W	1700	1·5	20·9	260
Bn772	Avisio Valley, Italy	46° 18′ N	11° 28′ E	950	−1·5	18·7	218
Dactylis glomerata							
Bc5655[c]	Pontevedra, Spain	42° 32′ N	8° 23′ W	365	6·9	19·2	365
Bc6684	Hattfjeldal, Norway	65° 35′ N	14° 00′ E	235	−9·0	13·1	122
Bc7070	Tjøtta, Norway	65° 50′ N	12° 25′ E	13	−0·6	13·3	150

[a] Lines were collected as seed by staff of the Plant Introduction Unit of IGER (formerly the Welsh Plant Breeding Station). Climate data were obtained from meteorological stations close to the collection point.

[b] Adapted British cultivar derived from Bn77, a population collected in Buckinghamshire, and an earlier variety, Bn95, of Scottish origin.

[c] Temperature records obtained from Santiago de Compostela, a more northerly station with comparable elevation and climate.

leaves of 10 separate plants were randomized, incubated on moist filter paper and exposed to a range of temperatures on a thermal gradient bar for 4 d as described by Thomas *et al.* (1980). Chlorophyll was extracted from 10 leaf segments in a total of 10 ml boiling ethanol and determined spectrophotometrically ($A_{660} \times 21{\cdot}0 = \mu g\ ml^{-1}$). The decolorised leaf tissue was air-dried, extracted for 20 h at 40 °C in 0·5 ml 1 M NaOH and protein determined in a 50 μl aliquot by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. Three replicates, each of 10 segments, were sampled at each temperature.

Curve-fitting and statistical analysis

Logistic curves were fitted to leaf length data using MLP (Maximum Likelihood Program – Ross, 1986), and Q_{10} and plastochron parameters were derived as described by Thomas & Potter (1985). The temperature sensitivities of chlorophyll (Chl) and protein (Prot) loss from senescing leaf sections were quantified by linear regression of $\log_e(S)$ on T for each genotype, where S = Chl or Prot content and T = temperature; Q_{10} was calculated as e^{-10k}, where k = slope of the regression line. Correlation coefficients were in the range 0·80–0·97. Parallel curve analysis in MLP was employed to verify differences.

Using MLP regression analysis, correlations between the Q_{10} values for corresponding physiological processes were determined ecotype by ecotype, to quantify how closely a given ecotype resembles each of the others in its overall sensitivity to low temperature. The Q_{10} for corresponding populations was also analyzed with respect to physiological process, to establish how consistently the responses of given processes remained in step (and thus possibly share temperature perception or transduction mechanisms) across the range of ecotypes.

RESULTS

Leaf extension curves

Leaf extension was quantified by fitting logistic-type curves to lengths measured for up to 120 d at 20 °C or after transfer to 5 °C at emergence of the fourth leaf. In all four *Fa* ecotypes, leaf 5 was observed to be consistently smaller than leaf 4 at full expansion (Fig. 1). Transfer to the lower temperature immediately constrained further extension of leaf 4 and resulted in a smaller asymptotic final length. The effect on leaf 5 was even more extreme, resulting in slow growth and much reduced lengths at full expansion.

Whereas the general pattern of extension and response to chilling temperature was similar for the four *Fa* ecotypes, detailed comparison of the primary and derived growth parameters obtained from the fitted curves revealed variations in behaviour between the lines (Table 2). Ecotype Bn772 was distinguished by its very long leaves and high mean absolute extension rates. By contrast the variety S170 grown at 20 °C had comparatively small leaves but a high mean relative growth rate. Consistent with these characteristics, the duration of leaf growth in S170 at 20 °C was relatively brief – about 2 wk, compared with the slow-growing Bn379, where D was about twice as long at the same temperature.

Ecotype Bn467 was particularly sensitive to low temperatures. Relative growth rates of leaves 4 and 5 were severely reduced and the duration of growth and length of plastochron greatly extended. A temperature of 5 °C appeared to be near a physiological threshold, resulting in increased within-genotype variation and high errors for Bn467. Likewise, S170 was quite severely limited by transfer to the chilling temperature and consequently some parameters are also statistically noisy.

Table 3 presents leaf sizes, growth rates and

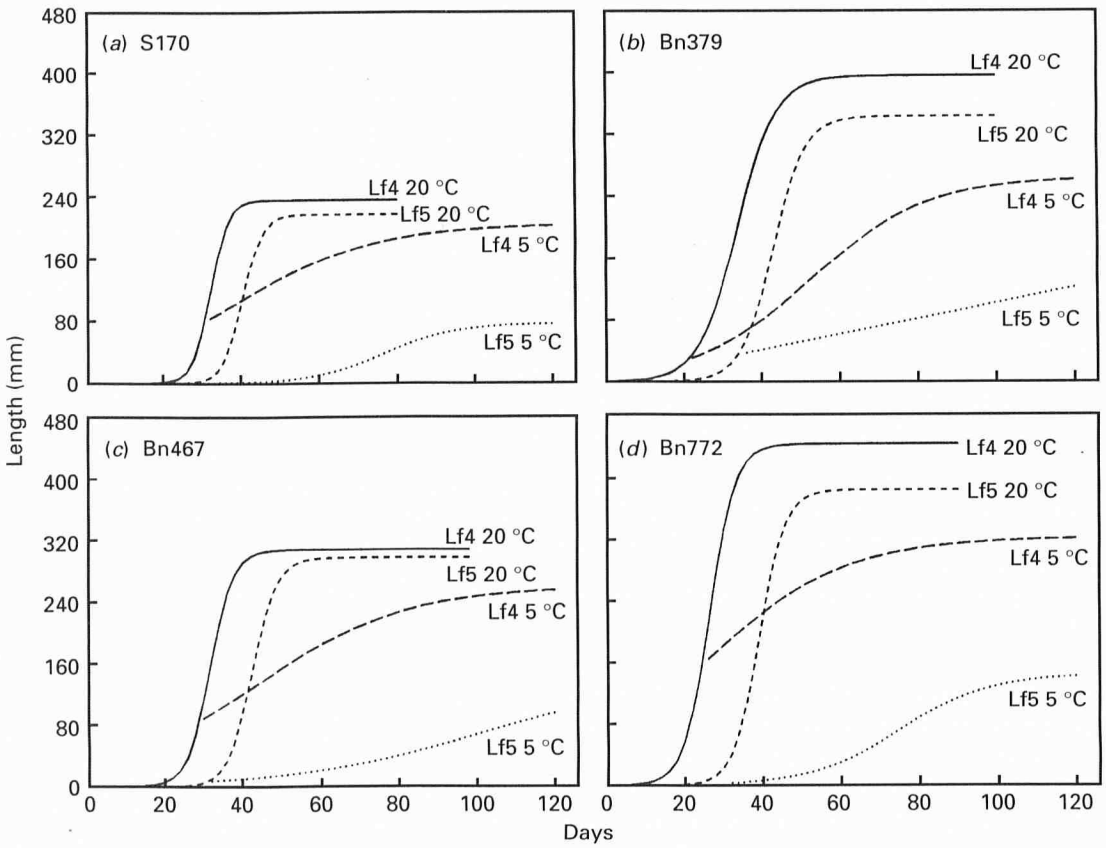

Figure 1. Logistic curves fitted to lengths of the fourth and fifth leaves of *Festuca arundinacea* ecotypes. Plants exposed to the 5 °C treatment were transferred from 20 °C at about the time of emergence of leaf 4.

Table 2. *Leaf growth parameters* (± *SE*) *for four* Festuca arundinacae *populations of contrasting geographical origin*

Ecotype/Leaf	°C	A (mm)	$\bar{R}$ (d^{-1})	$\bar{G}$ (mm d^{-1})	D (d)	P (d)
S170						
4	20	237·1 ± 6·7	0·22 ± 0·03	17·2 ± 2·0	13·8 ± 1·8	
	5	205·7 ± 11·8	0·028 ± 0·005	1·94 ± 0·24	105·9 ± 16·7	38·3
5	20	218·5 ± 16·2	0·20 ± 0·05	14·4 ± 2·9	15·2 ± 3·8	8·2
	5	77·6 ± 9·3	0·053 ± 0·028	1·38 ± 0·61	56·3 ± 28·2	
Bn379						
4	20	393·6 ± 37·2	0·10 ± 0·03	13·4 ± 3·6	29·3 ± 9·6	
	5	261·9 ± 29·5	0·034 ± 0·009	2·93 ± 0·58	89·4 ± 22·7	33·7
5	20	341·4 ± 106·0	0·12 ± 0·07	13·9 ± 4·2	24·6 ± 13·1	9·2
	5	145·9 ± 12·5	0·052 ± 0·02	2·51 ± 0·82	58·2 ± 21·3	
Bn467						
4	20	309·3 ± 8·1	0·17 ± 0·02	17·5 ± 1·7	17·7 ± 2·0	
	5	260·4 ± 32·5	0·026 ± 0·009	2·25 ± 0·55	115·6 ± 36·3	58·3
5	20	298·8 ± 32·1	0·16 ± 0·04	16·1 ± 2·7	18·6 ± 4·3	10·7
	5	139·1 ± 77·2	0·021 ± 0·014	0·95 ± 0·22	146·0 ± 85·8	
Bn772						
4	20	443·4 ± 20·3	0·15 ± 0·03	21·9 ± 3·2	20·3 ± 3·5	
	5	321·1 ± 9·9	0·029 ± 0·004	3·08 ± 0·33	104·4 ± 12·7	49·9
5	20	383·6 ± 16·8	0·15 ± 0·02	19·4 ± 2·2	19·8 ± 2·8	12·7
	5	144·7 ± 12·8	0·044 ± 0·011	2·13 ± 0·38	68·0 ± 15·3	

Leaf lengths of plants maintained at 20 °C or 5 °C were fitted to the logistic function and primary and derived parameters were estimated as described by Thomas & Potter (1985). A, final leaf length; $\bar{R}$, mean relative extension rate; $\bar{G}$, mean absolute extension rate; D, duration of growth; P, length of plastochron.

Table 3. *Leaf growth parameters (±SE) and temperature sensitivities of growth rates in three contrasting* Dactylis glomerata *populations*

	A (mm)		$\bar{R}_L$ (d^{-1})			$\bar{G}$ (mm d^{-1})			D (d)		
Ecotype	20°	5°	20°	5°	Q_{10}	20°	5°	Q_{10}	20°	5°	Q_{10} (R_{DW})
Bc5655	252±28	221±14	0·24±0·11	0·028±0·007	4·19	20·1±7·5	2·10±0·40	4·51	12·5±5·6	105±23	2·03
Bc6684	304±22	160±9	0·32±0·10	0·061±0·032	3·02	24·5±6·5	3·28±1·60	3·82	12·4±4·0	48·8±24·3	3·80
Bc7070	213±11	183±6	0·22±0·05	0·038±0·006	3·22	15·9±3·0	2·36±0·34	3·62	13·4±3·0	79·5±12·8	2·23

Mean absolute growth rate ($\bar{G}$) and relative growth rate ($\bar{R}_L$) were estimated from logistic curves fitted to leaf lengths against time. R_{DW} is the relative growth rate determined by destructive dry weight measurement.

Table 4. *Temperature responses over the range 20° to 5 °C, expressed as Q_{10}, of leaf growth rates in* Festuca arundinacea *populations of different geographical origins, derived as described in Table 3*

	$\bar{G}$		$\bar{R}_L$		
Ecotype	Leaf 4	Leaf 5	Leaf 4	Leaf 5	R_{DW}
S170	4·29	4·77	3·95	2·42	4·12
Bn379	2·76	3·12	2·05	1·75	1·77
Bn467	3·93	6·59	3·50	3·87	2·52
Bn772	3·69	4·36	2·99	2·27	2·59

durations for *Dg* ecotypes. The most prominent feature was the relative insensitivity to low temperature of final leaf length in Bc5655, though neither relative nor absolute extension rates were especially high at 5 °C in this line. Instead the growth period was prolonged at the lower temperature.

Temperature sensitivities of growth parameters

To place the thermal responses implied by Tables 2 and 3 on a quantitative footing, Q_{10} values for the mean absolute and relative extension rates of leaves 4 (*Fa*, *Dg*) and 5 (*Fa*) were computed (Tables 3, 4). The insensitivity of Bn379 to the step down in temperature was evident from the consistently low Q_{10} for both leaves and growth indices (Table 4). Further growth of leaf 4, already partially established at 20 °C, was most severely constrained by chilling in S170. By contrast, extension of leaf 5, the entire development of which occurred at 5 °C, was most sensitive to chilling in Bn467. Values of Q_{10} associated with mean absolute and relative extension rates for Bc5655, in which leaf size was almost unaffected by transfer to 5 °C, were much greater than those of the other two *Dg* ecotypes (Table 3) and were comparable with the most sensitive *Fa* lines and processes (Table 4). Relative growth rates were also estimated from dry weight measurements (Tables 3, 4). The comparative insensitivity of Bn379 and responsiveness of S170, expressed as Q_{10}, were

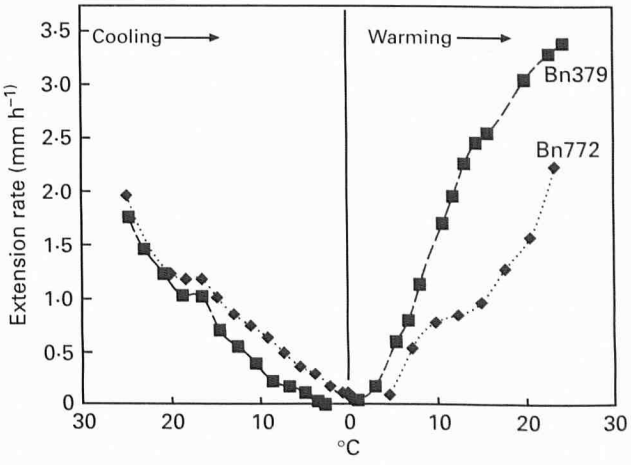

Figure 2. Extension rates of leaves of two *Festuca arundinacea* ecotypes in response to localized cooling and rewarming of the growing zone, measured with an electronic position transducer.

consistent with the picture given by $\bar{R}_L$ and $\bar{G}$. By contrast, Bc5655 was the least reactive of the *Dg* ecotypes when evaluated this way (Table 3).

Short-term growth responses

An electronic position transducer was employed to observe rapid adjustments in leaf elongation rate in response to changes in the temperature experienced by the growing zone in the region of the shoot intercalary meristem. Leaves were measured during the active growing phase between emergence from the sheaths of preceding leaves and the approach of full expansion. Figure 2 shows representative profiles for two of the *Fa* ecotypes. During the cooling cycle the extension rate declined until it became undetectable at the stop temperature (Table 5). The system was held at just below 0 °C for 20 min and the warming cycle was initiated. Table 5 presents temperature sensitivities and stop/restart temperatures, computed from fitted linear regressions. Ecotype Bn467 is particularly prominent in its low thermal sensitivity and relatively high threshold for growth cessation and restart. On the other hand the high Q_{10} values for Bn379 and Bc7070 show them to have been particularly responsive to growth constraint by chilling. The low start and (particularly)

Table 5. *Rapid temperature responses of leaf elongation rates in* Festuca arundinacea *and* Dactylis glomerata *determined using a temperature-profiled position transducer*

	Cooling		Warming		
Ecotype	Q_{10}	Stop T°	Q_{10}	Start T°	H
S170	1·59	4·24	2·23	2·65	11·6
Bn379	2·37	4·23	2·58	0·59	24·7
Bn467	1·54	6·28	1·59	4·30	3·8
Bn772	1·77	−0·26	2·47	0·86	4·7
Bc5655	2·63	3·90	2·06	0·55	3·8
Bc7070	3·21	5·47	2·48	3·25	14·9

Linear regressions were fitted to the cooling and rewarming curves and parameters computed from slopes and intercepts. All regressions were highly significant (correlation coefficients in the range 0·92–0·98).

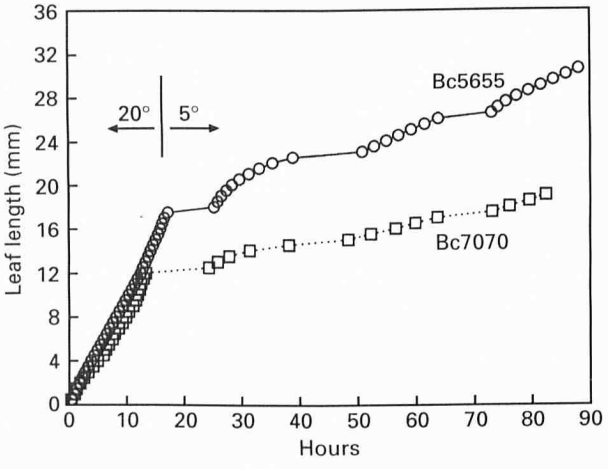

Figure 3. Lengths of growing leaves of two *Dactylis glomerata* ecotypes before and after transfer from 20 to 5 °C, measured with an optical auxanometer.

stop temperatures of Bn772 were especially notable. The parameter H is a measure of hysteresis, which was displayed by all genotypes to a greater or lesser degree (Fig. 2; Table 5). Ecotypes Bn467, Bc5655 and Bn772 represented one extreme whereas Bn379 showed strongly enhanced growth on rewarming.

Short-term modulations in extension growth conditioned by changing temperatures experienced by the growing zone are more complex than the data of Figure 2 and Table 5 suggest, because there might be interactions with the light environment. Thomas & Stoddart (1984) used an optoelectronic growth transducer in controlled environments to show that whereas *Lolium temulentum* leaves extended faster in the light phase of a 16 h dark/8 h light photoperiod at 20 °C, at 2 °C there was virtually no growth in the light and all measurable extension was confined to the dark period. A similar pattern was observed in the present study. Figure 3 shows that transferring Bc5655 or Bc7070, growing at roughly equivalent rates, from 20 °C to 5 °C resulted in a rapid

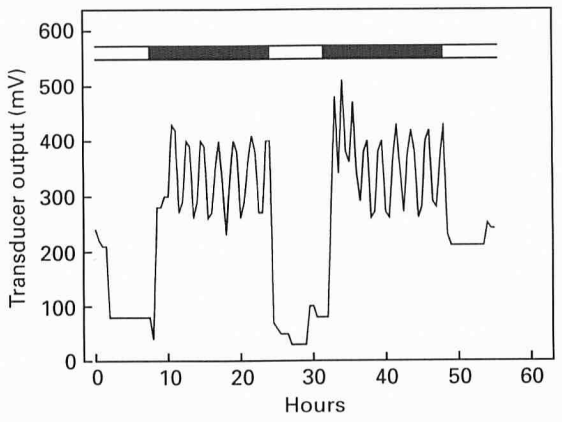

Figure 4. Auxanometer traces showing extension activity of leaves of *Dactylis glomerata* ecotype Bc5655 in relation to photoperiod. From one voltage maximum to the next is equal to 0·5 mm of growth. The alternating open and shaded bars represent the 8 h photoperiod and 16 h dark phase respectively.

readjustment. Growth of the Scandinavian ecotype Bc7070, was constrained to a greater degree than that of the Mediterranean race Bc5655. During the period at the lower temperature the extension profiles had the appearance of a series of rising curves and intervening plateaux (Fig. 3). Detailed examination of the optoauxanometer output traces (Fig. 4) revealed that little or no growth registered in each light period. Each of the LVDT experiments described in Figure 2 and Table 5 took place over a period of a few hours in the open laboratory with a constant low but uncontrolled ambient light environment of perhaps 150 μmol m^{-2} s^{-1}. Recent experiments with this equipment indicates that light-temperature interactions over short time scales might be more significant in quantifying thermal behaviour than considered hitherto (A. P. Gay & Henry Thomas, unpublished).

Chlorophyll and protein stabilities

Thomas *et al.* (1980) observed that senescence of excised leaf tissue was a useful physiological measure of the thermal behaviour of spring and winter oats. Segments of *Fa* and *Dg* leaves were incubated in darkness on a thermal gradient bar giving a range of temperatures between 0 and 20 °C, and Chl and Prot were determined after 4 d (Fig. 5). Loss of these components was relatively slow at temperatures up to about 8–10 °C and between this temperature and about 15 or 16 °C the extent of degradation was enhanced. In all cases the response was effectively modelled by fitting a declining exponential curve, the equivalent of first order kinetics with respect to 'thermal time'. The temperature sensitivities of Chl and Prot breakdown were quantified by Q_{10} (Table 6). A striking feature of these data is the uniformly low sensitivity of protein degradation. Q_{10} was well below 2. By contrast, values in excess of 4 were

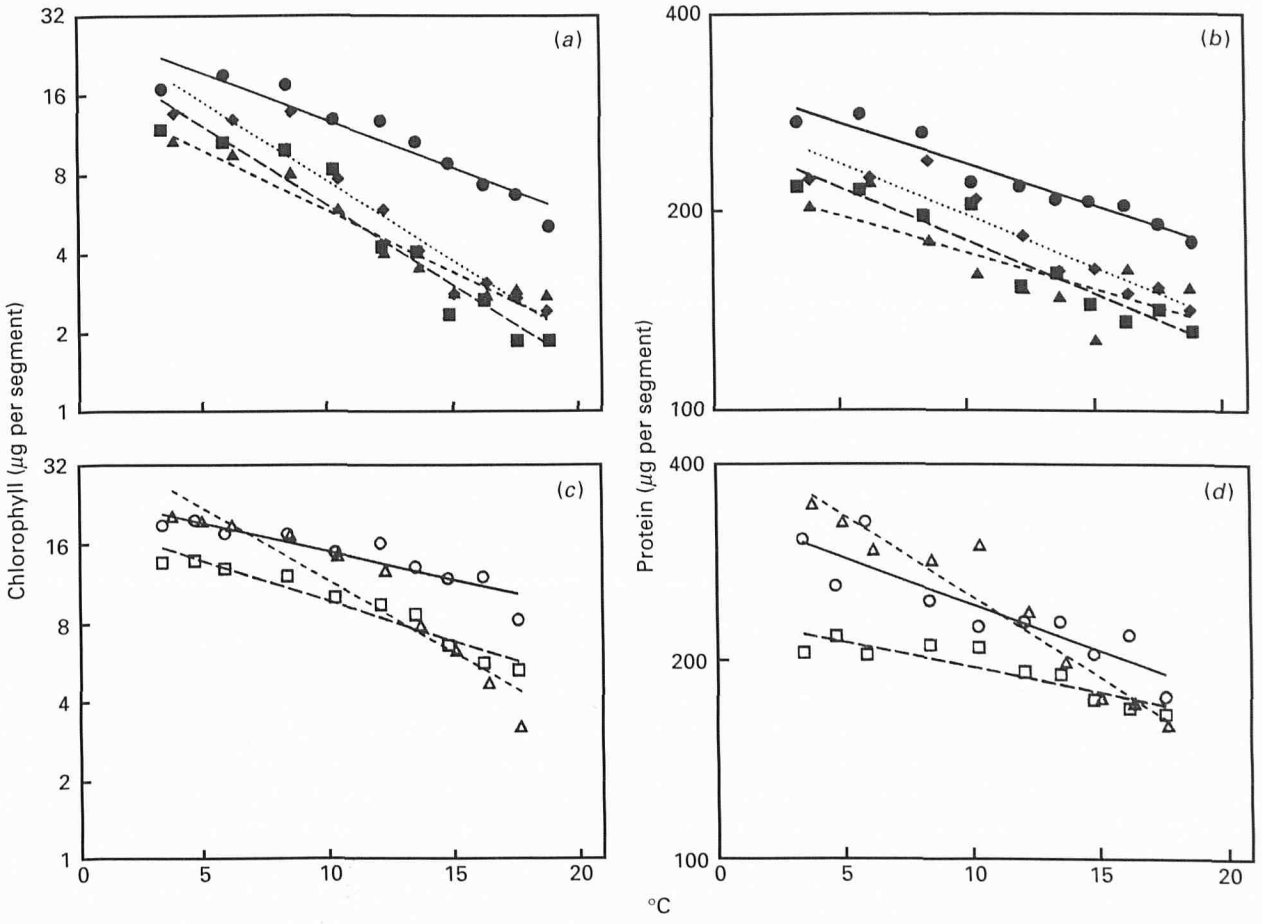

Figure 5. (*a*) Chlorophyll and (*b*) protein contents of *Festuca arundinacea* leaf segments senescing *in vitro* for 4 d at various temperatures in the range 3–20 °C. (*c*), (*d*) Chlorophyll and protein of leaves of *Dactylis glomerata* ecotypes. The lines represent linear regressions of $\log_e$(Chl) or $\log_e$(Prot) on temperature. ●, S170; ■, Bn379; ▲, Bn467; ◆, Bn772; ○, Bc5655; □, Bc6684; △, Bc7070.

Table 6. *Temperature responses of chlorophyll and protein degradation (Cd, Pd respectively) in leaf segments of* Festuca arundinacea *and* Dactylis glomerata *populations of different geographical origin.*

Ecotype	*Cd*	*Pd*
S170	2·31	1·34
Bn379	4·06	1·46
Bn467	2·93	1·30
Bn772	4·03	1·44
Bc5655	1·65	1·39
Bc6684	2·04	1·20
Bc7070	3·60	1·77

Data are Q_{10} values computed from slopes of linear regressions of $\log_e$(Chl) or $\log_e$(Prot) on temperature.

observed for Chl loss from tissue of Bn379 and Bn772. Bc5655 was the only ecotype in which Chl and Prot retained some kind of coordinated behaviour over the temperature range.

Correlations between ecotypes and Q_{10}

By bringing together the observations made in this study, it is possible to draw up a profile of thermal behaviour for each ecotype. Correlation analysis allows profiles to be compared and general similarities or disparities between ecotypes to be determined. Moreover, analysing correlations between physiological processes across all genotypes identifies those functional aspects that might have related temperature perception mechanisms. The grids of Table 7 compare ecotypes and processes pairwise with respect to Q_{10}; consistent responses to temperature are indicated by correlation coefficients in excess of about $\pm 0{\cdot}80$. Highly significant correlations were observed between Bc6684 on the one hand, and S170 and Bn467 on the other. The thermal behaviour of Bc7070 correlated strongly with that of Bn379 and of Bn772. Significant associations were also evident between Bn467 and Bc5655, and between Bn467 and Bn772. The combination of Bn379 and Bn467 was distinguished by a very low correlation coefficient, as were Bn379/Bn6684 and Bn379/S170. Interestingly, the relationships between ecotypes were almost completely non-transitive; that is, a particular ecotype might be highly correlated with two others – Bn6684 with S170 and with Bn467, for example – that are themselves not significantly related (Table 7).

Table. 7. *Correlation coefficients of* Q_{10} *values for leaf growth and senescence processes in* Festuca arundinacea *and* Dactylis glomerata *populations estimated from orthogonal regression analyses. Mean absolute growth rate* ($\bar{G}$) *and relative growth rate* ($\bar{R}_L$) *were estimated from logistic curves fitted to leaf lengths against time.* R_{DW} *is the relative growth rate determined by destructive dry weight measurement.* Cool *and* Warm *refer to, respectively, reducing and increasing temperature phases of measurement cycles using a growth transducer.* Cd *and* Pd *are chlorophyll and protein as indices of leaf senescence.* **, *Significant at* $P<0.05$; *, *significant at* $P<0.10$

	S170	Bn379	Bn467	Bn772	Bc5655	Bc6684				0·99 **	$\bar{R}_L$
Bc7070	0·43	0·88 **	0·72	0·95 **	0·60	0·65			0·51	0·53	R_{DW}
Bc6684	0·98 **	−0·02	0·88 **	0·41	0·37			0·59	−0·13	−0·22	Cool
Bc5655	0·76	0·47	0·82 *	0·43			0·45	−0·15	−0·58	−0·57	Warm
Bn772	0·44	0·80	0·85 *			0·58	0·07	−0·33	−0·89 **	−0·86 **	*Cd*
Bn467	0·79	0·00			0·44	0·60	0·84 **	−0·36	−0·30	−0·36	*Pd*
Bn379	−0·03				*Cd*	Warm	Cool	R_{DW}	$\bar{R}_L$	$\bar{G}$	

A similar analysis of correlations between processes (Table 7) revealed, not surprisingly, a very close relationship between mean absolute and mean relative growth rates and these were also negatively correlated with Chl degradation rates. An unexpected relationship between Prot degradation in senescence, and growth limitation as the meristem is cooled, was also suggested by these data.

DISCUSSION

Adaptation of leaf extension to low temperatures

Based on agronomic studies, complex models of the environmental control of leaf growth in *Fa* have been devised (see for example McCarty, Haun & Miller, 1991); but in general, under conditions where nitrogen supply is not limiting, the rate of extension of leaf tissue is principally a function of temperature (Gastal, Bélanger & Lemaire, 1992). Assimilate partitioning (Schnyder & Nelson, 1989; Allard & Nelson, 1991), nitrogen nutrition (MacAdam, Volenec & Nelson, 1989; Gastal & Nelson, 1994) and the interaction between them (Volenec & Nelson, 1984; Bélanger, Gastal & Warembourg, 1992) directly influence the division and expansion of leaf cells; temperature might alter growth indirectly by modifying any or all of these factors. On the other hand, high-resolution measurements of growth responses to temperature in grasses point to demand rather than supply processes as the point at which constraint is imposed (Thomas *et al.*, 1989). Over a longer period this feeds back into altered carbon and nitrogen partitioning. The mismatch between supply of raw materials and their utilization for growth is also well described for *Dg* (Eagles, 1967*a*, *b*; Pollock, 1982).

The occurrence of extension rate hysteresis during a rapid cooling–rewarming cycle (Stoddart *et al.*, 1986; Fig. 2; Table 5) and the pulse of growth immediately following lights off at the end of the light phase of the photoperiod at 5 °C (Thomas & Stoddart, 1984; Figs 3, 4) suggest that growth capacity also builds up in the very short term at suboptimal temperatures. Nevertheless the expectation of consistency between the temperature sensitivities of growth measured over the range of time scales, from plastochrons down to fractions of a second, was not met in the present study (Table 7). The factor responsible for detaching trends in the thermal responses of plastochron-scale extension rates from those of growth modulated by rapid temperature cycling might be acclimation. It is well established in chilling- and freezing-tolerant species that relatively fast initial adjustments to suboptimal temperature are followed by cold hardening – a gradual physiological realignment in which temperature optima are generally revised downward (Pollock & Eagles, 1988; Howarth & Ougham, 1993).

Influence of temperature on leaf senescence

A notable correlation revealed by the analysis presented in Table 7 is that between Chl and long-term mean growth rates. In general, the leaves of ecotypes in which growth is relatively sensitive to chilling are likely to be greener at suboptimal temperatures than those of insensitive lines because of an intrinsically higher stability of Chl across the temperature range. The correlation between Q_{10} for the Chl and Prot of *Fa* and *Dg* ecotypes was not significant (Table 7). It is known that the link between Chl and Prot degradation during leaf senescence is frequently quite tight under near-optimal environmental conditions but is highly fragile under stress (Thomas *et al.*, 1980; Mae *et al.*, 1993; Thomas & Smart, 1993). The relationship

between the Q_{10} for Prot and that for the cooling phase of LVDT measurement (Table 7) is unexpected. It might be significant that Pearce, Hawthorn & Ryle (1990) found a reduction in the proportion of newly-assimilated radiocarbon allocated to the protein fraction of growing shoots of *Fa* S170 transferred from a 17/14 °C light/dark environment to 7/4 °C. Protein turnover seems to be particularly sensitive to temperature and might well represent the biochemical link between Prot degradation during senescence of excised leaf tissue and rapid adjustments of extension rates under the influence of chilling.

Growth and senescence in relation to geographical origins of populations

Long-term growth behaviour, analysed by curve-fitting to leaf lengths, identified those ecotypes from the most severe habitats as generally the most temperature-sensitive. Thus the duration of the growing season in the habitat to which *Dg* ecotypes were adapted ranked consistently with the extent to which final leaf length was reduced by exposure to 5 °C (Table 3). The picture was not so clear for *Fa*, but the northern type S170 and, particularly, the high-altitude population Bn467 were more temperature-sensitive than was Bn379, the ecotype of Tunisian provenance from a location with a near year-round growing season (Table 1). The Italian population, Bn772, represented the main inconsistency. Although its habitat of origin has a relatively short growing season and experienced sub-zero temperatures in the coldest month (Table 1), with the exception of its long leaves, this ecotype was not particularly extreme in any of its growth parameters (Tables 2, 4). Generalizing, with the exception of Bn772, populations from environments with growing seasons most severely curtailed by low temperature respond to chilling by producing smaller, greener leaves. But differences are apparent between such populations in the means by which this reduction in leaf size is achieved. The Q_{10} for mean relative and absolute growth rates were greater for the Spanish ecotype of *Dg* than for the Norwegian lines; but the duration of growth was even more sensitive to chilling, and was more influential than were $\bar{R}$ and $\bar{G}$ in determining the rank order of leaf size adaptation (Table 3). On the other hand, the relatively high sensitivity of leaf size to low temperature in *Fa* S170 and Bn467 is most consistently associated with high Q_{10} for growth rate parameters (Table 4). In general, therefore, reduced leaf size at chilling temperatures is associated with slower rates of growth in *Fa* and a shorter growth period in *Dg*.

Correlations between processes and populations

As discussed above, the temperature sensitivities of short-term growth processes do not relate to those of growth over days or weeks in any very simple way. Consequently, consistencies between ecotype provenances and thermal behaviour quantified by growth transducer measurement are difficult to discern. The Spanish *Dg* population Bc5655, in which leaf growth is persistent under chilling conditions, had lower Q_{10} and threshold temperatures than the chilling-responsive Norwegian ecotype Bc7070 (Table 5). Auxanometry showed that growth rates were also less constrained in Bc5655 than in Bc7070 on transfer from 20 °C to 5 °C (Fig. 3). The pattern of reactions of *Fa* populations to rapid cooling and rewarming did not reveal a convincing relationship to provenance (Table 5). On the whole, the *Fa* ecotypes behaved in a much more varied and inconsistent fashion with respect to profiles of temperature sensitivities than did the *Dg* populations. In each case where there was a significant overall correlation, the ecotypes in question were of different species – Bc6684 with S170, Bc707 with Bn772 and so on (Table 7). Correlation and principal component analysis of within-species variation in morphology and physiology has identified distinct ecotype groups within *Fa* (Ueyama & Sato, 1994) and *Dg* (Fujimoto, 1993). It is precisely this degree of variability, with its underlying wealth of unlinked genetic diversity, that makes adapted populations from different climates such a valuable resource for improving the tall fescue and cocksfoot crops.

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