Analysis of the temperature effect on the components of plant digestibility in two populations of perennial ryegrass

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Abstract: For the development of mechanistic models of herbage digestibility, quantitative insight into the effects of age, temperature and cultivar on digestibility characteristics of individual plant parts is needed. Towards that goal, glasshouse experiments were conducted at day/night temperatures of 13/8, 18/13 and 23/18 °C with vegetative and reproductive crops of two populations of perennial ryegrass (Lolium perenne L) selected for differences in leaf blade digestibility. Cell wall content (CWC) and true cell wall and organic matter digestibility (CWD and OMD) of vegetative and reproductive tillers were related to dimensions, mass, CWC and digestibility of separate plant parts. Compared with the vegetative tillers, the reproductive tillers had higher rates of leaf appearance, organic matter growth and CWD decline. Strikingly, for both tiller types, no direct effect of temperature on whole tiller CWD was observed, since temperature effects could be eliminated completely by relating CWD to development stage (DVS) expressed as number of leaves appearing on the main tiller. Temperature effects on CWD were restricted to its influence on tiller development rate only. The decline of CWD of individual plant parts with DVS in the reproductive tillers could be described with a negative exponential curve, which reached an asymptote that was higher for leaf blades (755 g kg⁻¹) than for leaf sheaths (491 g kg⁻¹) and stem internodes (230 g kg⁻¹). However, all plant parts in both tiller types had the same fractional CWD decline rate of 0.395 per leaf appearance interval, independent of plant part insertion level, population or temperature. Differences between temperature treatments in OMD were caused by the higher CWC of plant parts at higher temperature, due to a stronger decline of the specific organic matter mass than of the specific cell wall mass of plant parts at increasing temperature. Differences in whole tiller OMD between populations were observed only for vegetative tillers and were also caused by differences in CWC. It is concluded that temperature increase accelerated both the tiller development rate and the rate of decline of CWD during aging to the same extent, whereas plant parts respond similarly in the fractional CWD decline pattern as a function of DVS. These trends offer unique possibilities for modelling grass digestibility under contrasting temperature regimes.

Keywords: perennial ryegrass; digestibility; aging; temperature; vegetative; reproductive; cell wall; development stage

INTRODUCTION

Temperature and reproductive development are the most important factors determining composition and digestibility of grass.¹ Temperature affects digestibility through changes in three plant characteristics: (i) the rate of development, (ii) the ratio between cell wall and cell contents and (iii) the rate of cell wall aging, which results in a decline of cell wall digestibility. The contribution of these three plant processes to the generally observed temperature effect has not been quantified before and will be analysed in this paper. The analysis is based on glasshouse experiments with vegetative and reproductive tillers of two populations of perennial ryegrass differing in leaf digestibility.

The digestibility differences could have been caused by contrasts in cell wall digestibility, but differences in development rates could also play a role.

This paper is a new contribution to an explanatory mechanistic analysis of changes in grass digestibility. In earlier papers the relationships between plant development and digestibility and the digestibility decline in consecutively formed individual leaves on the main shoot of Italian ryegrass were studied.²,³ An important parameter related to plant development that will also be used here is the leaf appearance rate.² The aging of cell walls in leaves (decline of digestibility) starts even before leaf appearance, during the elongation of the leaf in the sheath tube.²,⁴ Therefore in...
reproductive tillers the changes in composition and digestibility of sheaths and internodes during their elongation also need to be quantified.

In the experiments described in this paper, changes in cell wall content and digestibility of vegetative and reproductive tillers of the two populations were quantified and related to the dimensions, mass, composition and digestibility of the plant parts, in particular the leaf blades of vegetative tillers and the leaf blades, leaf sheaths and stem internodes of reproductive tillers. Since temperature is the most important environmental factor that influences digestibility of grasses,1 plants were grown under three temperature regimes.

**EXPERIMENTAL**

**General**

Two experiments with vegetative (experiment 1) and reproductive (experiment 2) perennial ryegrass were carried out in three well-controlled glasshouses with day/night temperatures of 13/8, 18/13 and 23/18°C. In both experiments, two ryegrass populations differing in leaf blade dry matter digestibility were included. Day temperatures were maintained between 08:00 and 20:00. Relative humidity was on average 70%. The experimental periods and average radiation intensities are summarised for both experiments in Table 1 (see ‘Results’). Radiation data were obtained from the nearby Wageningen weather station. Daily irradiance was corrected for the transmission coefficient (0.7) of the greenhouse.

Seeds were sown into pots containing a mixture of sand and peat (1:1 v/v). The pots were placed in large trays, which were flushed with mineral solution5 daily. Seeds of two populations of perennial ryegrass (*Lolium perenne* L), differing in leaf blade digestibility (population 1, high; population 2, low), were used (seed kindly supplied by Barenbrug BV, Oosterhout, The Netherlands). Plants were harvested five times, based on development stage. Four replicate blocks were placed in each glasshouse. Within the blocks, 10 rows of six pots were randomly assigned for each population × harvest time combination. Border pots were placed adjacent to the experimental pots. Harvested material of replicate blocks 1&2 and 3&4 was bulked to give two replicates for chemical analyses of individual leaf blades, leaf sheaths and stem internodes.

**Experiment 1. Vegetative tillers**

In March 1993, pots for the three temperature regimes were seeded at weekly intervals, so that plants would reach the four-leaf stage on the main tiller (ligule leaf 4 appeared) at approximately the same moment. Day length was natural. Plant density was 20 per dm². Plants were cut at 3.5 cm above ground level in the four-leaf stage. Harvests were made at each leaf appearance interval from leaf stage 6 onwards. Consecutive leaf blades from the main tiller were dissected and analysed.

**Experiment 2. Reproductive tillers**

In September 1993, seeds were sown and placed in an unheated glasshouse for vernalisation during winter. Plant density was 15 per dm². Plants were cut on 28 October 1993 at the four-leaf stage. In March 1994, pots were transferred to heated glasshouses with day/night temperatures of 13/8°C. For the 18/13 and 23/18°C regimes the temperature was increased by 1°C day⁻¹ until the required temperatures were reached, in order to avoid loss of vernalisation. Moreover, day length was extended to 17 h by low-radiation (25 W) light bulbs to initiate inflorescence development and stem elongation at the same moment at every temperature. In the early stages of the experiment, two harvests were taken for determination of whole crop characteristics. Thereafter, for analyses of individual plant parts, plants were harvested at the moment of flag leaf appearance and after one and two intervals of 21, 14 and 10 days thereafter for the 13/8, 18/13 and 23/18°C temperature regimes respectively. From each pot the 15 largest tillers were selected and dissected into separate leaf blades, leaf sheaths and stem internodes. Leaves and internodes were numbered from the top to the bottom of the tiller, starting with the first leaf formed after transfer (= leaf 1).

**Measurements**

In both vegetative and reproductive plants the time of appearance of leaves on the main tiller was recorded to determine the rate of tiller development. The leaf appearance rate was used to calculate the development stage of tillers (Sₜ, in leaf appearance intervals). The age of plant parts (Sₗ) was expressed in leaf appearance intervals after their full expansion. Although in reproductive tillers no new leaves are formed after flag leaf appearance, the leaf appearance rate before that stage was used to calculate Sₗ and Sₜ. Two harvests were made before flag leaf appearance. These were made at different development stages in the three temperature treatments, because the development stage relative to the flag leaf was not known before its appearance.

The length and width of leaf blades and stem internodes were recorded. Leaf area was calculated from length and width using a calibration factor to correct for leaf shape.6 The leaf sheaths in the vegetative plants were not analysed, because these form only a minor part of the animal diet. In a reproductive crop, leaf sheaths are elevated along with the internodes and thus will be harvested, at least with silage cuts.

Samples of consecutive leaf blades, sheaths and stem internodes were oven dried for 24 h at 70°C and subsequently weighed to determine the dry matter (DM) content. Samples were ground to pass a 1 mm screen in a hammer mill and analysed for cell wall content in organic matter (CWC) using neutral detergent solution.7 Undissolved protein was removed by incubation of the cell wall residue with alcalase.
(0.75 ml in 40 ml of buffer solution) for 1 h at 40 °C. Cell wall digestibility after 48 h (CWD) was measured using an incubation of 0.5 g of air-dry matter in rumen fluid from fistulated steers fed medium-quality grass hay. True organic matter digestibility (OMD) was calculated assuming complete digestibility of the cell contents. From the mass of organic matter (OM; dry matter minus ash) and CWC (g kg⁻¹ OM), cell wall (CW) mass was calculated. From the data on area, length, OM mass and CW mass, specific masses of OM and CW were calculated for each plant part (mg cm⁻² for leaf blades and mg cm⁻¹ for leaf sheaths and stem internodes).

Data analysis

To determine the effects of development stage, temperature and population on OMD, CWD and CWC of whole tillers and on CWD and specific OM and CW mass of plant parts, stepwise multiple regression analyses were carried out using Genstat. Terms included in the models for whole tiller composition were temperature (T), population (P), replicate (R), development stage (Sₜ), S₂ₜ and interaction terms (full model \((Sₜ + S₂ₜ) \times T \times P \times R\)). For the plant parts the age was expressed in leaf appearance intervals after full expansion (Sₗ), and a term for insertion level (l) was included in the model, which resulted in the full model \((Sₗ + S₂ₗ) \times T \times P \times l \times R\). Terms were added to the model provided they resulted in a significant \((p < 0.05)\) reduction in the residual mean square.

The curves of decline of CWD of individual plant parts were fitted non-linearly with a negative exponential decline curve:

\[
\text{CWD}_{Sₗ} = z + (1000 - z)e^{-\beta(Sₗ - \lambda)}
\]

In this equation, \(z\) denotes the asymptotic digestible cell wall content (g kg⁻¹), \(\beta\) the fractional rate of decline of CWD (per leaf appearance interval), \(Sₗ\) the development stage expressed in leaf appearance intervals after full plant part expansion, and \(\lambda\) the development stage at which CWD decline commences. Since synthesis of indigestible cell wall starts before full expansion, \(\lambda\) has a negative value of about \(-1\)².

RESULTS

Tiller growth and development

Individual reproductive tillers showed considerably higher OM growth rates than vegetative tillers (Table 1). Development rates were higher at higher temperature for both tiller types and, only for reproductive tillers, these rates were higher in population 1 than in population 2. The accelerated development rate at higher temperature was reflected in (i) shorter growth periods for reaching the chosen development stage of the last harvest, ie two leaf appearance intervals after flag leaf appearance in the reproductive tillers, and (ii) the increased leaf appearance rate with temperature (Table 1). At final harvest, stem internodes and ears formed about 75% of total harvested shoot OM in the reproductive tillers of both populations (data not shown).

Specific mass of plant parts

In the vegetative tillers the leaf blades of consecutive insertion levels had higher specific OM and CW masses, whereas the specific OM mass declined after ligule appearance at more or less similar rates for the three leaf blades (Fig 1(A)). Contrastingly, the leaf blade specific CW mass remained constant after ligule appearance. Also in the reproductive tillers the leaf blades of consecutive insertion levels had higher specific OM mass (Fig 1(B)). After flag leaf appearance the specific OM mass of lower leaf blades declined, but it increased slightly for the flag leaf (L6). The leaf blade specific CW mass remained constant after flag leaf appearance (Fig 1(B)). The sheath of the flag leaf (L6) and the youngest stem internodes (L5 and L6) were not fully grown at flag leaf appearance and continued to increase in specific OM and CW mass, while the specific CW mass of the sheaths and internodes of lower insertion levels remained unchanged (Figs 1(C and D)). The final specific CW mass of leaf sheaths was higher for consecutive insertion levels (Fig 1(C)), while the specific

<table>
<thead>
<tr>
<th>Tiller type</th>
<th>Temperature (°C)</th>
<th>Period (day of year)</th>
<th>Radiation (MJ m⁻² day⁻¹)</th>
<th>Growth rate (mg OM per tiller day⁻¹) P1</th>
<th>Growth rate (mg OM per tiller day⁻¹) P2</th>
<th>Lₗ (day⁻¹) P1</th>
<th>Lₗ (day⁻¹) P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>13/8</td>
<td>129–214</td>
<td>11.8</td>
<td>1.7</td>
<td>1.7</td>
<td>0.0362</td>
<td>0.0360</td>
</tr>
<tr>
<td></td>
<td>18/13</td>
<td>127–176</td>
<td>12.2</td>
<td>3.5</td>
<td>4.3</td>
<td>0.0637</td>
<td>0.0615</td>
</tr>
<tr>
<td></td>
<td>23/18</td>
<td>128–155</td>
<td>12.5</td>
<td>3.8</td>
<td>3.7</td>
<td>0.0927</td>
<td>0.0824</td>
</tr>
<tr>
<td>R</td>
<td>13/8</td>
<td>149–192</td>
<td>12.6</td>
<td>10.7</td>
<td>9.4</td>
<td>0.0804</td>
<td>0.0696</td>
</tr>
<tr>
<td></td>
<td>18/13</td>
<td>129–157</td>
<td>10.9</td>
<td>10.6</td>
<td>8.5</td>
<td>0.1271</td>
<td>0.1089</td>
</tr>
<tr>
<td></td>
<td>23/18</td>
<td>112–136</td>
<td>10.9</td>
<td>10.4</td>
<td>7.8</td>
<td>0.1607</td>
<td>0.1429</td>
</tr>
</tbody>
</table>

Table 1. Experimental periods and average daily incoming solar radiation corrected for the transmission coefficient (0.7) of the glasshouse, and average rates of tiller OM growth and leaf appearance (Lₗ) for vegetative (V) and reproductive (R) perennial ryegrass populations 1 and 2 (P1 and P2) grown at three temperatures.
CW mass of stem internodes seemed to converge to the same level for all internodes (Fig 1(D)).

Whole tiller composition and digestibility

A stepwise multiple regression analysis was carried out to establish the most important trends in composition and digestibility of whole tillers (Table 2). In the vegetative tillers, about 75% of the variation in OMD was explained by development stage (factors $S_T$ and $S_T^2$). In the reproductive tillers the contribution of development stage was even higher, explaining about 90% of the variation in OMD. Consequently, the direct effect of temperature on OMD was small (less than 10%) in both tiller types. OMD declined with development stage (Figs 2(E and F)) and was slightly lower (vegetative tillers; Fig 2(E)) or decreased faster (reproductive tillers; Fig 2(F)) at higher temperature. Variation in CWD was almost exclusively explained by development stage in both tiller types (Table 2 and Figs 2(A and B)). This implies that temperature had no direct effect on CWD. Therefore all of the variation in OMD due to temperature differences could be explained by the effects of temperature on CWC. In the vegetative tillers, more than 50% of the variation in CWC was explained by temperature (Table 2). It can be seen in Fig 2(C) that CWC increased with development stage in the 18/13 and 23/18°C treatments and decreased slightly in the 13/8°C treatment,
Significance: a, p<0.001; b, p<0.01; c, p<0.05.

Table 2. Results of regression analysis for organic matter digestibility (OMD), cell wall digestibility (CWD) and cell wall concentration (CWC) of whole vegetative and reproductive tillers versus development stage (S): contribution of significant terms to $r^2 (%)$ of the regression model, and total $r^2$ (%) of the final model

<table>
<thead>
<tr>
<th>Term $^a$</th>
<th>Vegetative OMD</th>
<th>CWD</th>
<th>CWC</th>
<th>Reproductive OMD</th>
<th>CWD</th>
<th>CWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>7.83a</td>
<td>1.68b</td>
<td>56.32a</td>
<td>0.66b</td>
<td>20.91a</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>4.21a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_1$</td>
<td>1.54a</td>
<td>82.14a</td>
<td>11.96a</td>
<td>88.94a</td>
<td>96.67a</td>
<td>59.19a</td>
</tr>
<tr>
<td>$S_2$</td>
<td>74.22a</td>
<td>12.44a</td>
<td>1.00a</td>
<td>8.15a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T \times P$</td>
<td>1.11b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$T \times S_1$</td>
<td>3.44a</td>
<td>6.19a</td>
<td>4.95a</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$T \times S_2$</td>
<td>1.30b</td>
<td></td>
<td>0.59b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P \times S_1$</td>
<td>1.50b</td>
<td>5.71a</td>
<td>0.33b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P \times S_2$</td>
<td>0.39b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T \times P \times S_1$</td>
<td>0.56b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r^2_{adj}$</td>
<td>89.4</td>
<td>84.8</td>
<td>89.2</td>
<td>96.6</td>
<td>96.9</td>
<td>94.2</td>
</tr>
</tbody>
</table>

$^a$ T, temperature; P, population; $S_1$, development stage; $S_2$, quadratic development stage.

resulting in significant $S_1$, $S_2$ and $T \times S_T$ terms (Table 2). Minor effects of population on OMD were present in the vegetative tillers only (Table 2 and Fig 2(E)). In the reproductive tillers, CWC increased before flag leaf ligule appearance ($S_1=6$) and faster at higher temperature (Fig 2(D)), which led to significant $S_1$ and $T \times S_T$ terms in the regression analysis (Table 2). However, CWC stabilised after flag leaf appearance and at higher levels at higher temperature (Fig 2D; $S_2$ and $T$ terms in Table 2).

The contribution of leaf blades to total tiller CW mass in leaf blades decreased slowly in the vegetative tillers (Fig 3(A)) but rapidly in the reproductive tillers (Fig 3(B)). In the reproductive tillers, stem internodes and ears together comprised about 70% of whole tiller CW mass (Figs 3(D and E)). The much faster decline in CWD and the faster increase in CWC in the reproductive tillers with development stage (Figs 2(B and D) respectively) were due to the formation of stem internodes (Fig 3(D)).

Digestibility patterns during aging
The pattern of decline of CWD with development stage after full plant part expansion ($S_1$) of leaf blades, leaf sheaths and stem internodes is illustrated for the reproductive tillers in the 18°C temperature treatment in Fig 4(A). The fitted curves were obtained with eqn (1). Leaf blades, leaf sheaths and stem internodes differed in asymptotic CWD (Fig 4(B)) but had the same fractional CWD decline rate of 0.395 per $S_1$ (Fig 4(C)). The asymptotic maximum was higher for leaf blades (755 g kg$^{-1}$) than for leaf sheaths (491 g kg$^{-1}$) and stem internodes (230 g kg$^{-1}$). The asymptotic CWD and the fractional CWD decline rate per $S_1$ of all plant parts in both tiller types (data not shown for reproductive tillers) were not affected by plant part insertion level, temperature or population.

Composition of plant parts
In all plant parts the specific masses of OM and CW were lower at higher temperature, but the relative reduction in specific OM mass was larger, resulting in higher CWC (Fig 5). CWC of stem internodes increased in the whole range of temperatures from 10 to 20°C (Fig 5(D)), while, in leaf blades and sheaths, CWC mainly increased from 10 to 15°C (Figs 5(A–C)).

Populations
Although in all plant parts there was a trend to a higher CWC in population 2 at all temperatures (Fig 5), regression analysis only showed a significant population effect on whole tiller CWC for the vegetative tillers ($P \times S_2$ interaction in Table 2). Consequently, only the vegetative tillers of population 2 had a lower OMD than population 1. The only significant difference in CWD between populations concerned leaf blades of reproductive tillers at 18°C, which showed higher CWD for population 2 than for population 1 (Fig 4(A)). However, this had no effect on whole tiller CWD (Fig 2(B)), because the leaf blade fraction in total CW mass was low (Fig 3(B)).

DISCUSSION
The observed differences between the vegetative and the reproductive tillers could not be analysed statistically, because the data were obtained from separate experiments. Furthermore, in the vegetative tillers, changes in leaf characteristics were measured after ligule appearance, whereas, in the reproductive tillers, these changes were monitored for all plant parts from the moment of flag leaf appearance onwards. Nevertheless, the contrasts between the two tiller types in morphological development, CWC and digestibility were very clear. The reproductive tillers had a higher leaf appearance rate and formed stem internodes. A higher leaf appearance rate and higher OM growth rates (Table 1) for reproductive tillers than for vegetative tillers are generally observed. The more rapid growth of reproductive tillers has been attributed to the cessation of root growth and tillering, remobilisation of reserves from the roots, and the enhanced photosynthetic rate in leaves of vernalised tillers. In both the vegetative and the reproductive tillers the specific CW mass remained constant for fully expanded leaf blades (Figs 1(A and B)), which is in accordance with earlier observations. In leaf sheaths the specific CW mass stabilised one leaf appearance interval later than in leaf blades, as far as measured in the reproductive tillers (Fig 1(C)). Stem internodes reached their final length and CW mass after approximately two leaf appearance intervals following ligule appearance of the leaf blade of the same
Figure 2. Cell wall digestibility (CWD: A, B), cell wall content (CWC: C, D) and organic matter digestibility (OMD: E, F) at 13/8 (T1), 18/13 (T2) and 23/18 (T3) °C of vegetative (A, C, E) and reproductive (B, D, F) tillers of populations 1 and 2 (P1 and P2) versus tiller development stage (S_T). Additional lines indicate the significance (p < 0.001) of temperature (full) and population (broken) terms in the regression model.
phytomer (Fig 1(D)). This corresponds to the coordinated pattern of tiller development described for tall fescue (*Festuca arundinacea* Schreb).\textsuperscript{15}

Important trends were found in CWD decline of plant parts. The CWD decline curves of not only the leaf blades but also the leaf sheaths and stem internodes in the reproductive tillers could be fitted satisfactorily with the same equation as used earlier for

![Graph A](image)

![Graph B](image)

![Graph C](image)

![Graph D](image)

![Graph E](image)

**Figure 3.** Contribution of various plant parts to total tiller cell wall mass versus development stage ($S_T$): leaf blades of vegetative tillers (A) and leaf blades (B), leaf sheaths (C), stem internodes (D) and ears (E) of reproductive tillers. Temperatures 13/8 (T1), 18/13 (T2) and 23/18 (T3) °C; populations 1 and 2 (P1 and P2).
the leaf blades of Italian ryegrass.\textsuperscript{2} The asymptotic CWD for both leaf sheaths and stem internodes was lower than for leaf blades (Fig 4(B)). As a consequence, the strong decline of the leaf blade fraction in whole tiller CW mass (Fig 3(B)) and the increase in the proportion of internodes and ears (Figs 3(D and E)) resulted in faster CWD decline \textit{versus} development stage in reproductive tillers (Fig 2(B)) than in vegetative tillers (Fig 2(A)). The asymptotic CWD of plant parts was not significantly affected by insertion level (Fig 4(A)) or temperature (Fig 4(B)). This is probably related to the virtually unchanged tissue proportions in the cross-sectional area of plant parts at contrasting temperatures.\textsuperscript{16–18}

Generally applicable relationships for modelling purposes obtained in this experiment concerned the relationship between temperature, and morphological development (leaf appearance) and cell wall digestibility. The rates of both leaf appearance and decline of CWD were enhanced at higher temperature, but to the same extent, so that the fractional CWD decline rate per leaf appearance interval was unaffected by temperature (Fig 4(C)). The absence of temperature effects on asymptotic CWD and fractional CWD decline rate of plant parts and the convergence between temperature treatments in the proportions of plant part types in the whole tiller CW mass (Fig 3) explain why the relation between tiller CWD and tiller development stage ($S_T$) decreased linearly for all treatments (Figs 2(A and B)).

Consequently, the effects of temperature on OMD have to be fully explained from differences in CWC. These can result from differences in the proportions between plant parts (leaf blade, sheath and stem internode, of which internodes have the highest CWC) or the differences in CWC in plant parts as a result of temperature. Since the contributions of plant part fractions to whole tiller CW mass converged for temperature treatments (Fig 3), the higher CWC of plant parts at higher temperature (Fig 5) was the sole cause of the temperature effect on OMD. Probably, the higher development rate and higher maintenance respiration rate at higher temperature resulted in a lower availability of assimilates per plant part formed on a tiller.

Also, the differences in whole tiller OMD between populations were limited and only significant in vegetative tillers (Table 2 and Fig 2(E)). As can be seen in Figs 2(A and C), they were caused only by differences in CWC. No significant relations with morphological characteristics were observed, although population 2 tended to have longer and narrower leaves and a lower leaf appearance rate (data not presented). It has been demonstrated that these characteristics can be correlated with digestibility.\textsuperscript{19,20} However, the correlations were never strong.

**CONCLUSIONS**

The present experiments revealed that temperature increase accelerated both the tiller development rate and the CWD decline rate during aging to the same extent, whereas plant parts responded similarly in CWD decline pattern as a function of development stage. These trends offer unique possibilities for modelling grass digestibility under contrasting tem-
perature regimes. Moreover, the rate of tiller development, ie leaf appearance rate, and the pattern of decline of CWD during aging of the various plant parts seem to be most appropriate to relate to herbage digestibility in breeding programmes.

REFERENCES
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