Developmental genetics of leaf formation in *Lolium*

1. BASIC PATTERNS OF LEAF DEVELOPMENT IN *L. MULTIFLORUM* AND *L. PERENNE*

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1. INTRODUCTION

During the early stages of the growth of a grass crop, the rate of growth will be largely limited by the size of the total leaf surface available to intercept incoming solar radiation. The faster this total leaf surface area is increased the faster will be the rate of growth of the crop. But once the leaf surface forms an almost complete canopy and intercepts nearly all the incoming radiation, the situation becomes more complex because of the shading of one leaf by another and because of leaf senescence. Even after this stage, there is likely to be some positive relation between the rate of formation of new leaf surface and the crop growth rate, if only because most of the material formed by the crop is leaf tissue. Expansion of leaf surface area will be of great importance in determining growth rate in the early seedling stage and after defoliation by grazing animals or mechanical cutting.

In studies of the inheritance of two of the principal components of rate of leaf surface formation, it was found that individual leaf size and rate of appearance of new leaves were negatively correlated genetically (Cooper & Edwards, 1961; Edwards & Cooper, 1963). Selection for either component produced a negative response in the other, and led to plants having either a slow production of large leaves or a rapid production of small leaves. Since such negative correlated responses occurred in all three populations studied, it appeared that they were an expression of a fundamental developmental relation between these two characters.

There exist a number of studies of the growth patterns of individual leaves in the Gramineae (e.g. Sharman, 1945; Williams, 1960; Soper & Mitchell, 1956). The general pattern emerging from these studies is that the growth of an individual leaf (either by volume or weight) can be divided into three phases:

(i) Primordial growth. From the inception, a primordium grows exponentially but slowly until it becomes longer than the apical meristem, and in fact forms a 'hood' over and around the meristem and the younger primordia.

(ii) Rapid growth. At some stage after it has overtopped the apex, the young leaf undergoes a sudden transition to a more rapid growth rate. This stage is again exponential but the exponent is greater.

(iii) Declining growth. As the leaf emerges and matures, the growth rate declines until eventually it ceases altogether.

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The transition between the first and second phase is fairly clear-cut, but that between the second and third phase is less well defined. Sharman (1945) related the first transition to the development of vascular connexions between the young developing leaf and the main shoot. Soper & Mitchell (1956) described the localization of cell division and enlargement within the tissues of a developing leaf of *Lolium perenne*. The meristematic activity of a growing leaf is confined to an intercalary meristem at the base of the leaf. The distal parts of the leaf mature first and an increase in the length of the leaf occurs because the mature parts are pushed up by the meristematic activity of the base.

Yamazaki (1963) has studied the relations between the stages of growth of successive leaves on a shoot in rice at any given time. He has shown that when an individual leaf has reached the 'hood' stage and is developing vascular connexions, the next older leaf is actively elongating but has not emerged, the next but one older leaf is elongating and has emerged, while the next but two older leaf is mature. Yamazaki's data refer to a static assessment of a particular point in the development of the shoot. In order to interpret the negative genetic correlations between leaf size and rate of leaf appearance reported in earlier papers, a dynamic picture is needed. In particular, it would be interesting to answer the question: are there any regular relationships between successive leaves on a shoot involving the timing of changes in phases of development of a single leaf, such as the transition to rapid growth and the complete cessation of growth? This paper is an attempt to provide such a dynamic picture of the interrelations between the patterns of growth of successive leaves on the main shoot of *Lolium*. Subsequent papers will deal with the interpretation of the responses of selection lines in terms of such a model.

2. MATERIALS AND METHODS

(i) *Materials*

Two species of ryegrass were used:

> *Lolium multiflorum* (Bb 1155, a commercial cultivar of Italian ryegrass).
> *Lolium perenne* (Ba 6738, a commerical cultivar of perennial ryegrass).

Although only one variety of each species was used, the varieties concerned differ from one another in leaf characters, as will be seen from the results, and are believed to be representative of their respective species. Both *L. multiflorum* and *L. perenne* are diploid and they are interfertile, but they have evolved in geographical isolation to an extent that they differ considerably in general morphology and in growth rhythm. The general morphology of vegetative plants of both species has been described by Cooper & Edwards (1961). *L. perenne* has smaller leaves (both shorter and narrower) than *L. multiflorum*.

(ii) *Experimental details*

Most of the data presented in this paper were collected from a glass-house experiment sown in July 1964. The plants were grown in soil in boxes. The experiment

was arranged in two blocks and at each dissection harvest groups of five plants of each species were taken at random from each box. Six dissection harvests were made at 16, 23, 30, 34, 38, and 42 days after sowing. Morphological measurements were made on twenty plants of each species.

A second sowing was made in January 1965 to compare patterns of leaf development at different seasons. This also was a glass-house experiment and the plants were grown in soil in boxes. There were three blocks, and morphological measurements were taken on five plants of each species per block.

(iii) *Measurements and specification of leaf growth patterns*

There are obvious problems in collecting data on the growth of primordia and of young leaves before they become visible. There are two possibilities. Firstly, serial dissections can be made from which growth patterns are reconstructed; but this technique suffers from two main disadvantages. One is that dissections involve destructive sampling, so that an entirely new sample must be used for the next harvest. This multiplies the size of the experiment and also introduces problems of sampling error in genetically heterogeneous populations like ryegrass. The other disadvantage is that it is impossible to measure directly the growth rate of an individual organ. Secondly, estimates of the early growth of a leaf might be made from direct measurements of the growth rate at the later, visible stage. The use of growth measurements, made in the visible period of a leaf's growth to make inferences about early growth, avoids destructive sampling but involves certain assumptions about uniformity of growth rates before and after emergence from the preceding leaf sheath. These assumptions will be considered later. In this study, the investigation involving the first experiment was carried out using both dissections and inferences from visible growth. Dissections were mainly used to check the validity of the assumptions.

The final area of an individual leaf will depend upon its length and width. It is difficult to measure changes in widths during the development of a leaf because the width of each segment of a leaf is determined before it emerges from the leaf sheath and, in fact, is very largely dependent upon the diameter of the shoot apex (Soper & Mitchell, 1956). The width is little modified during the development of the leaf. Final leaf length, on the other hand, is clearly going to be modified by the rate and duration of activity of the intercalary meristem. For these reasons this study is mainly concerned with the rate and duration of elongation of leaves.

The measurements actually made on the visible growth of leaves were:

  (i) Date of appearance of tip of leaf from sheath of preceding leaf, D_A.
 (ii) Total length of leaf at D_A, measured as sum of visible length (L_v) plus overall length of tube formed by sheaths of older leaves (L_s), L_A.
(iii) Total length of leaf 4 days after appearance of tip, $L_{(A+4)}$.
 (iv) Date at which leaf ceases growth, D_M.
  (v) Total length of leaf at maturity, L_M.
 (vi) Length and width of mature blade, L and W respectively.

From these records the following additional metrics were derived:

(vii) Date of 'unfolding' of leaf, $D_0 = D_A - \dfrac{4L_s}{L_{(A+4)} - L_A}$.

The term 'unfolding' is used to indicate the onset of rapid elongation of a leaf, following the terminology of Milthorpe (1959).

(viii) Rate of leaf elongation immediately after appearance, $R = \dfrac{L_{(A+4)} - L_A}{4}$.

  (ix) Duration of elongation of a single leaf, $\text{Dur} = D_M - D_0$.

The estimation of the date of onset of leaf growth, D_0, involves certain assumptions. These are:

1. That the apical meristem is situated at the base of the tube formed by the mature leaf sheaths, i.e. L_S represents the distance a leaf has to grow before it appears.
2. That the rate of leaf elongation, as directly measured immediately after appearance, is a measure of the rate immediately before appearance and that the rate is, in fact, linear over a long period of growth.

In an attempt to check these assumptions, at each dissection harvest in the July-sown experiment the lengths of all leaves longer than the apex, the total number of leaves longer than the apex, and the number of visible primordia on the apex were recorded.

3. RESULTS

(i) *Validity of assumptions*

The two assumptions listed in the previous section concerning the making of inferences from externally measured leaf growth data can be tested by comparing estimates derived in this way with those obtained from dissection. The first assumption, that the leaves are borne at the apical meristem at the base of the tube formed by existing leaf sheaths, was found by direct observation to be valid.

The second assumption concerned the linearity of the rate of elongation before emergence and immediately after. This can be tested indirectly by comparing, for specific leaves, the estimated date of onset of growth (D_0) with observation from dissections of the stage of development of the leaves at given dates. Several observations from the dissection data, such as the number of leaves longer than the apex or the number longer than 1 mm., were compared with the leaf growth estimates. The closest correlation with the latter was given by the number of leaves longer than 1 mm., as can be seen in Fig. 1. The correlations are very close to linear ones and the regression and correlation parameters are shown in Table 1. The agreement between the two methods can be seen by comparing for each species the predicted Y values for $X = 30$ from the respective regression coefficients, where $X =$ the number of days from sowing and $Y =$ the number of leaves which have unfolded on the main shoot. For *Lolium multiflorum* the two values are 6·67 and 6·65, for *L. perenne* they are 5·85 and 5·79.
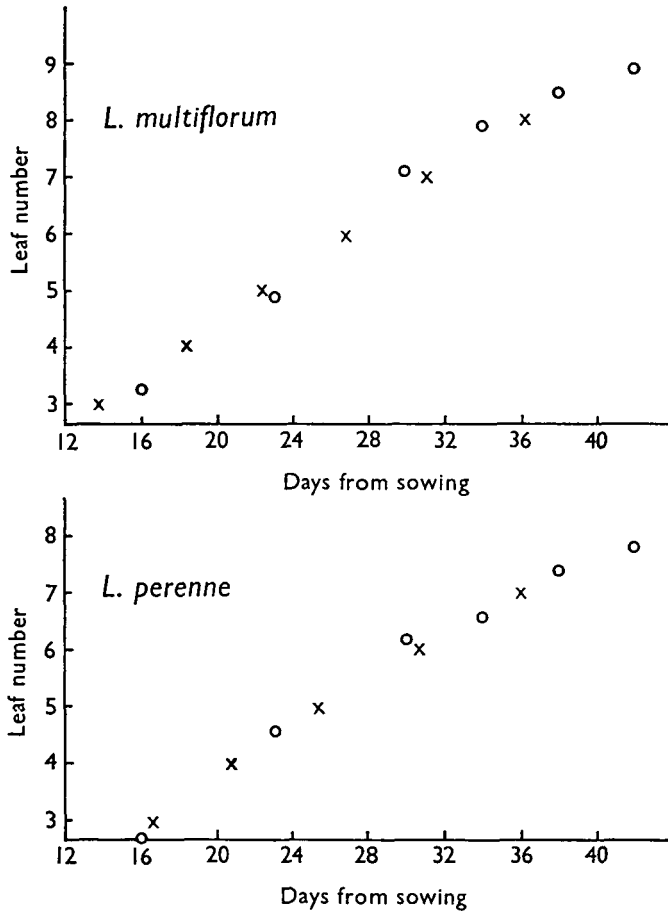
Fig. 1. Comparison of dates of leaf unfolding in *Lolium multiflorum* and *L. perenne* as estimated from:

(a) × Morphological data on leaf elongation.

(b) ⊙ Data from dissections of shoot apex.

Thus it seems that the use of visible leaf elongation data to estimate the onset of the phase of rapid leaf elongation is reasonable and that in fact the transition from slow primordial growth to rapid elongation occurs when the leaf is approximately

Table 1. *Onset of unfolding. Comparison of estimates from visible leaf data and from dissections. July sowing*

Species	Estimate from	Regression equation	s_b	r
Lolium multiflorum	Leaf growth	$Y = -0.08 + 0.225X$	0.006	0.999
	Dissection	$Y = -0.13 + 0.226X$	0.018	0.987
Lolium perenne	Leaf growth	$Y = -0.27 + 0.204X$	0.005	0.999
	Dissection	$Y = -0.10 + 0.196X$	0.015	0.988

X = Number of days from sowing.
Y = Number of unfolded leaves.

16

1 mm. long, at least for the first eight or nine leaves on the main shoot. This being so, it was decided that this method could be used in the present studies, and would be advantageous for two reasons. Firstly, it allows larger samples to be taken, which is desirable in an outbreeding and highly variable plant such as ryegrass. Secondly, it allows the growth of successive leaves on the same plant to be followed. The greater precision of this method over the dissection method can be seen from a comparison of standard errors in Table 1.
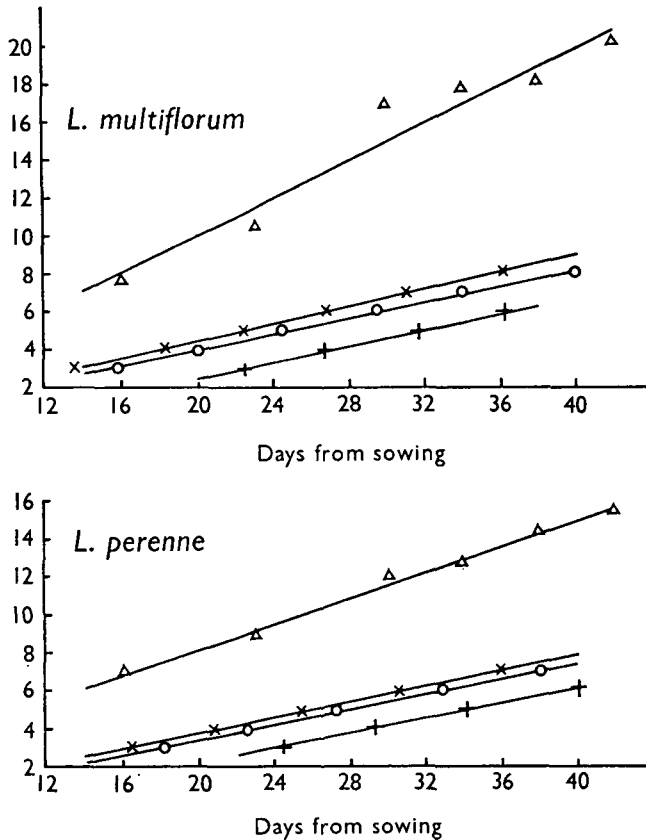

Fig. 2. Rates of leaf development in *Lolium multiflorum* and *L. perenne*:

△ Primordia initiation
× Leaf unfolding
○ Leaf appearance
+ Leaf maturation.

(ii) *Rates of formation of new leaves*

The rate at which successive leaves on the shoot reach comparable growth stages can be defined in various ways according to the criterion of growth stage chosen. Four definitions are used in this study. These are:

(i) Rate of primordia formation. The rate at which primordia are produced at the shoot apex, as determined from dissections.

(ii) Rate of leaf unfolding. The rate at which successive leaves reach the onset of the phase of rapid elongation.

(iii) Rate of leaf appearance. The rate at which the tips of successive leaves become visible.

(iv) Rate of leaf maturation. The rate at which successive leaves mature.

The results for all four rates are presented in Figs. 2a and 2b. The regression coefficients in Table 2 are in terms of leaves per day. These data are from the July-sown experiment. The January experiment showed a very similar pattern of results although the absolute values differed. The rates of primordia formation are much faster than the other rates ($P < 0.001$). This causes the characteristic build-up of primordia on the apex. The build-up is very marked in *L. multiflorum* where 20 days after sowing there are five or six primordia visible on the apex, but at 40 days after sowing this number has risen to eleven. The other three rates in each species are very similar, but the mean of the three in *L. multiflorum* (0.216 leaves per day) is significantly greater ($P < 0.01$) than that for *L. perenne* (0.198 leaves per day). The rate of primordia formation in *L. multiflorum* is also greater ($P < 0.05$) than that in *L. perenne*. The rate of leaf unfolding is somewhat greater than the rates of appearance and maturation, averaged over both species ($P < 0.05$). This difference appears to be an artefact of the experiment. All three rates were declining slightly with time and since they were all measured on the same leaf intervals, the rate of unfolding was in fact measured rather earlier in time than the other rates.

Table 2. *Rates of formation of leaves* (*leaves per day*)

	L. multiflorum	*L. perenne*
Rate of primordia formation	0.494 ± 0.061	0.337 ± 0.015
Rate of leaf unfolding	0.225 ± 0.006	0.204 ± 0.005
Rate of leaf appearance	0.208 ± 0.004	0.199 ± 0.006
Rate of leaf maturation	0.215 ± 0.008	0.192 ± 0.008

(iii) *Duration and rates of leaf elongation*

The duration of elongation of individual leaves can be calculated from the data in Fig. 2 as the interval between the time of unfolding and the time of maturation and this is done in Table 3. The same data are also presented in Fig. 3 in order to show the relations between the times of unfolding and maturation of successive leaves. To do this is revealing because it shows that there is a very close association between the maturation of any leaf, say (*n*), and the unfolding of the next leaf but one (*n* + 2). The association is almost completely synchronous for both species in both the July and the January experiments, although in *L. perenne* in the July experiment there is a small but positive, and just significant ($P < 0.05$), delay between the two events while in *L. multiflorum* in the January experiment there is a slight and non-significant overlap. Since leaves in grasses are borne alternately on opposite sides of the shoot, it appears that the time of unfolding of a leaf is very closely related to the time of maturation of the next older leaf on the same side of the apex.

Table 3. *Duration of leaf elongation. July sowing*

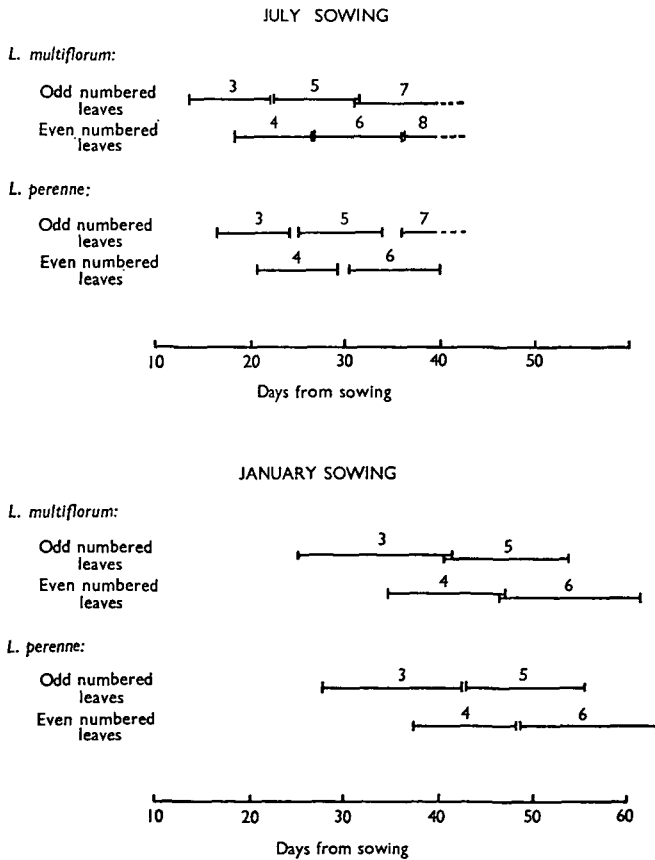|	L. multiflorum			L. perenne		
	Date of			Date of		
Leaf number	Unfolding	Maturation	Duration (days)	Unfolding	Maturation	Duration (days)
3	13·7	22·4	8·7	16·5	24·4	7·9
4	18·4	26·7	8·3	20·7	29·3	8·6
5	22·4	31·7	9·3	25·4	34·1	8·7
6	26·9	36·2	9·3	30·6	40·1	9·5
7	31·1	—	—	36·0	—	—
8	36·2	—	—	—	—	—
Standard error	0·38		0·22	0·51		0·22

Fig. 3. Timing and duration of growth of successive leaves of *Lolium multiflorum* and *L. perenne* in July and January experiments.

The rates of elongation of leaves are given in Table 4. There is a large difference between the means of the two species averaged over all leaves ($P < 0.001$). There is also significant variation between leaves within each species. The rate tends to rise with successive leaves until leaf 6, and then to fall off at leaf 7.

Table 4. *Rates of leaf elongation (mm./day)*

Leaf number	L. multiflorum		L. perenne	
3	25·0 ⎫		17·5 ⎫	
4	34·1 ⎬ ± 1·16		19·3 ⎬ ± 0·76	
5	32·7 ⎭		20·8 ⎭	
6	33·5 ⎫		23·3 ⎫	
7	27·9 ⎭ ± 1·65		21·8 ⎭ ± 1·08	
Mean	30·6	± 0·55	20·5	± 0·41

(iv) *Mature leaf length*

Final leaf length and its components, blade length and sheath length, are given in Table 5. In both species there is an ontogenetic trend for increased length. This occurs as a result of increases in both blade and sheath, although the proportionate increase of the latter is greater. For leaf 3 the sheath accounts for approximately 12% of the total length, but for leaf 6 this proportion has risen to 18%. The trend for increased length up to leaf 6 appears to be due in part to increased duration of elongation and in part to increased rate.

Table 5. *Leaf lengths (mm.)*

	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Standard error
L. multiflorum					
Total length	184	223	264	301	10·8
Blade length	162	192	221	247	10·0
Sheath length	22	31	43	54	2·4
L. perenne					
Total length	119	141	173	196	4·7
Blade length	103	120	142	161	4·6
Sheath length	16	21	31	35	0·8

(v) *Comparison of species*

The differences between *L. multiflorum* and *L. perenne* are summarized in Fig. 4 in which the lengths of individual leaves are plotted against time throughout the duration of the July experiment. *L. multiflorum* had a more rapid rate of leaf formation, eight leaves having appeared by the end of the experiment (42 days after sowing) compared with seven leaves in *L. perenne*. The rates of leaf elongation are greater in *L. multiflorum*, as was noted in Table 4. The duration of growth of individual leaves differs little between the two species, but *L. multiflorum* leaves are longer because of the faster rate of elongation.
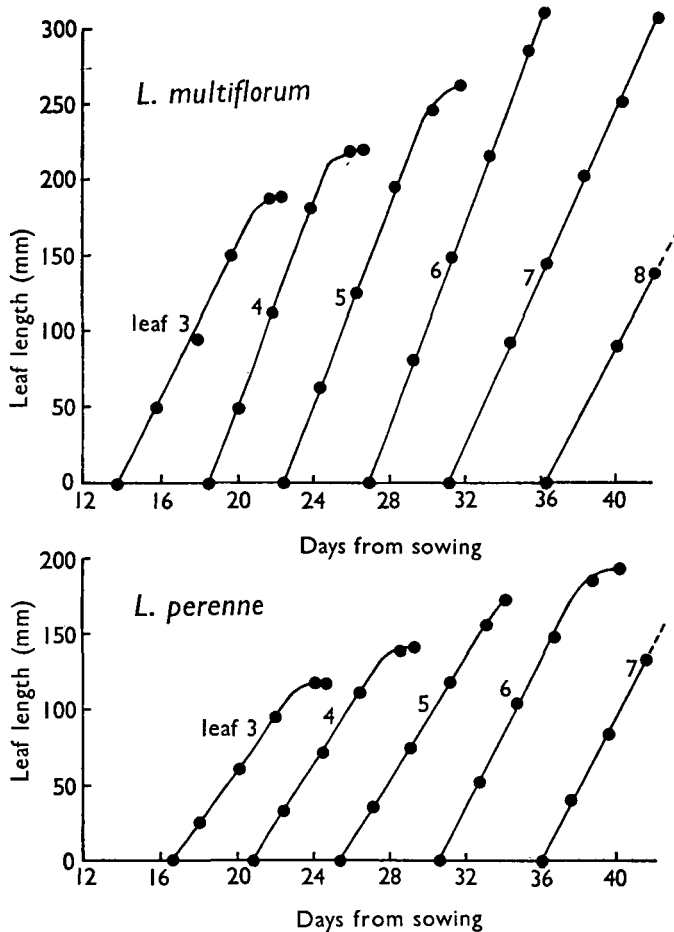
Fig. 4. Rates of leaf development in *Lolium multiflorum* and *L. perenne*.

(vi) *Comparison of sowing dates*

Most of the data reported so far were obtained from the July experiment. The relative pattern of leaf development in the two species was very similar in the January experiment, although the absolute values of the various parameters of development changed. Table 6 summarizes these changes.

The rate of leaf unfolding is lower in the January experiment (as were also the rates of appearance and maturation) in both species, though it is again a little higher in *L. multiflorum* than in *L. perenne*. The duration of leaf elongation is considerably greater in January and there are only very slight differences between species in either experiment. The rate of leaf elongation is greatly reduced in January while in *L. multiflorum* it remains much higher than in *L. perenne*. This reduction more than compensates for the increased duration so that the leaf is finally much shorter in January although here again the difference between the species is very similar in both environments.

Table 6. *Comparison of leaf development at two sowing dates*

Species:	*L. multiflorum*		*L. perenne*	
Sowing date:	January	July	January	July
Character				
Rate of leaf unfolding (lvs/day)	0·147 ± 0·020	0·225 ± 0·006	0·140 ± 0·017	0·204 ± 0·005
Duration of elongation leaf 6 (days)	15·1 ± 0·70	9·3 ± 0·22	14·6 ± 0·36	9·5 ± 0·22
Rate of elongation of leaf 6 (mm./day)	17·6 ± 0·48	33·5 ± 0·55	12·4 ± 0·45	23·2 ± 0·41
Final length of leaf 6 (mm.)	235 ± 5·7	301 ± 12·9	167 ± 3·8	196 ± 6·9

4. DISCUSSION

The fairly abrupt increase in the growth rate of a young developing leaf, which other workers have reported in Gramineae, appears to occur in ryegrass when the young leaf is about 1 mm. long. During the course of the July experiment the length of the apex ranged from 0·29 to 0·61 mm. in *Lolium multiflorum* and 0·23 to 0·44 mm. in *L. perenne*. Thus the transition in growth rate occurred when the primordium had grown about two or three times as long as the apex. Esau (1954), Sharman (1945) and Williams (1960) have shown in other Gramineae that the vascular system of the developing leaf becomes connected to the main bundles in the stem at about the time when the young leaf is forming a hood over the apex, and is seems very likely that such a development is responsible for the sudden increase in leaf growth rate in ryegrass at this stage.

One of the major aims of this investigation has been to determine whether there are any close relationships between the stages of development of successive leaves. To answer this question, Fig. 3 has been prepared. It shows that the transition from slow primordial growth to rapid leaf elongation, which I have called the time of unfolding, of a given leaf occurs more or less simultaneously with the maturation of the next but one older leaf. Since the leaves are borne alternately on opposite sides of the apex, the unfolding of a leaf is synchronous with the maturation of the next older leaf on the same side of the stem. The mechanism responsible for this association is not known. It could be that a young leaf is inhibited from unfolding by the active expansion of the next older leaf on that side of the apex. Alternatively, the unfolding of a leaf may be responsible for cessation of expansion of the older leaf by competition for substrates. The data do not allow a distinction to be made between these two hypotheses.

Whatever the cause the fact of the association remains and it is consistent over both species and both environments. This consistency suggests a fairly fundamental and well-canalized relationship which is unlikely to be modified by selection for a character expressed at a later stage of development, such as leaf size or rate of leaf appearance. Thus one would predict that a response to selection for leaf size, in so

far as it is achieved as a result of an increase in the duration of leaf elongation, is likely to be accompanied by an increase in the interval between the unfolding of successive leaves, which will lead to reduced rates of leaf unfolding and leaf appearance. Conversely a response to selection for faster rate of leaf appearance will almost certainly involve a faster rate of leaf unfolding which in turn will be expected to reduce the duration of leaf elongation and thereby shorten the leaf and reduce its size.

In principle, an increase in leaf size could occur as a result of greater width with no change in length. If the response to selection for leaf size is mainly through width, this would be unlikely to have an effect on rate of leaf appearance. The chances of such a response occurring without a concomitant increase in length will depend on the genetic correlation between width and length. Cooper & Edwards (1961) showed that this genetic correlation was positive and large in *L. multiflorum* but small and not significantly different from zero in *L. perenne*. But even if an increase in length does occur as a response to selection for leaf size, it does not necessarily follow that the duration of leaf elongation has increased. It could have been a result of a faster rate of elongation alone, and if so there would be no expected change in rate of leaf appearance. Again the probability of such a change in rate of elongation occurring independently of duration will depend on the genetic correlation, of which the present data provide no estimates. The phenotypic correlation has been estimated for leaves 4 and 6 in the July experiment. The coefficients are small and non-significant for leaf 4 in both species and for leaf 6 in *L. perenne* although positive ($+0.63$) and significant ($P < 0.01$) in *L. multiflorum* for leaf 6. These varying estimates suggest that a response in rate of leaf elongation independently of duration of elongation is a possibility; in fact such an independent change occurs between *L. multiflorum* and *L. perenne*.

The predictions of responses to selections for either leaf size or rate of leaf appearance can be summarized as follows on the basis of the model of leaf formation developed in this paper. Selection for rate of leaf appearance would be expected to be regularly accompanied by a reduction in individual leaf area, but selection for larger leaves may or may not be accompanied by a slower rate of leaf appearance depending on whether or not a change in duration of leaf elongation has occurred. Edwards & Cooper (1963) have shown that negative correlated responses between leaf size and rate of appearance do occur in selection lines. The extent to which the details of these responses fulfil the predictions made in this paper will be examined in the following paper in this series.

SUMMARY

1. Repeated measurements on the growth of individual leaves in seedlings and young plants of ryegrass combined with dissections of the apex of the shoot and of very young leaves have shown that the basic pattern of leaf formation is very similar in *Lolium multiflorum* and *L. perenne*.

2. The initial growth rate of a leaf primordium is low but increases suddenly at a

point when the primordium is somewhat longer than the apex and about 1 mm. in length. After this transition, which I have called the unfolding of the leaf, the rate of elongation is faster and more or less linear until the leaf is nearly mature.

3. The time of unfolding of a leaf is very closely associated with the time of maturity of the next older leaf on the same side of the apex. Thus a leaf ceases growth when the next younger leaf immediately above it starts elongating rapidly, though which is cause and which is effect is it not possible to say.

4. This close relationship between duration of leaf elongation and rate of unfolding of successive leaves holds for both species in two seasons. It leads one to predict that selection for increased leaf size, in so far as it is a result of greater duration of leaf elongation, is likely to be accompanied by a slower rate of leaf appearance, and conversely that selection for rate of leaf appearance is likely to result in smaller leaves.

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17