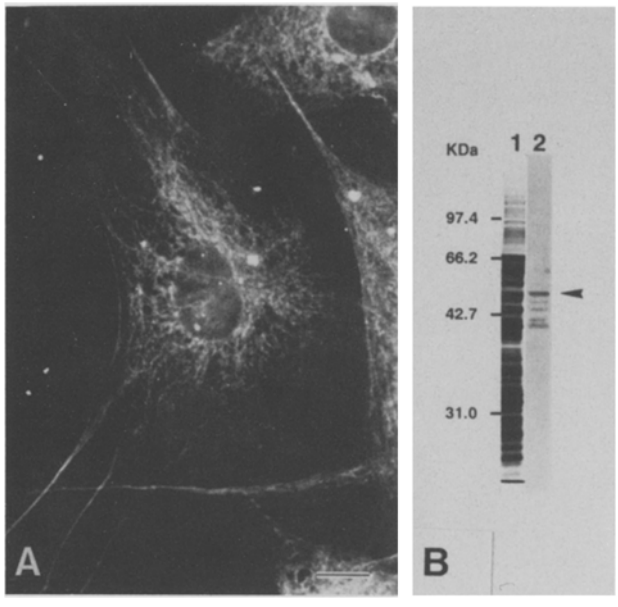

Figure 6. *A* Immunocytochemical demonstration of desmin. Scale bar = 10 µm. *B* Immunoblot analysis of proteins of XTY cells. Each lane loaded with 5 µg proteins. Lane 1: silver stain of polyacrylamide gel. Lane 2: immunoblot of anti-desmin antibody. Arrow points to 52 kD protein known to be desmin.

XTY cells also did not demonstrate any intercellular junctions and expressed desmin but not keratin. We therefore suggest that this cell line derives from interstitial rather than epithelial cells.

Immunoblot analysis shows desmin, which is a major protein of myogenic cells [10]. Minor bands seen in lane 2 of figure 6 might be degraded or nonspecific protein bound to contaminant antibodies contained in the primary antibody. Our results suggest that the XTY tumor cells are myogenic interstitial cells.

XTY cells form large aggregates which are different from the focus generally formed by transforming cells. The diameter of the aggregates is medium-sized (more than 2 mm). Some mitotic cells are present in the inner parts of aggregates; therefore, we consider that the cells within the aggregates do not undergo necrosis. This is also indicated by our finding that aggregates transferred to a new flask expand and proliferate. We believe that aggregated XTY cells represent a differentiated form of the XTY cells after confluency. When an aggregate (2 mm in diameter) was transplanted under the skin of the back of an adult *Xenopus laevis* tumor formation did not result.

In conclusion, the new cell line XTY has immortalized, and forms aggregates, but shows no malignancy. Morphology and ultrastructural features as well as the expression of desmin lead us to suggest that XTY cells are not epithelial, but interstitial. We plan to compare the XTY cell line with other *Xenopus* cell lines in order to determine its specific characteristics.

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Leaf-blade crimping in grasses: A new measure of growth

P. R. Espie, H. E. Connor[a] and I. J. McCracken

Forest Research Institute, P.O. Box 31-011, Christchurch (New Zealand), and [a] Centre for Resource Management, University of Canterbury, Christchurch (New Zealand)
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Abstract. In grasses, ligules compress and permanently crimp emerging leaf-blades (primary crimping). Ligule compression may also mark the abaxial surface of older leaves in some species (secondary crimping). Secondary crimping appears directly related to leaf daily growth rate. Leaf-blade crimping can be used for determination of relative tissue age and as a natural record of growth rate in grasses.
Key words. Grasses; crimping; leaf; leaf-blade; ligule; growth rate; *Chionochloa.*

Assessment of growth rate is fundamental to grassland ecological studies[1]. Here we report leaf crimping for the first time and explain its origin. It offers a new way of measuring growth.

The shape of grass leaf-blades often changes slightly, particularly near the apex. Flat leaf-blades become distorted or narrowed, sometimes with a groove or ridge across them, then re-expand below the constriction. In terete, conduplicate, or involute blades the constriction can be a longer narrow zone. We call this 'crimping' as blades are compressed, pinched, or indented.

As far as we are aware this feature has not been described previously in grass morphology[2–10], apart from a passing observation in wheat[11]. Crimped leaf-blades are common in the genera we examined (table 1), and we expect crimping to be nearly universal in grasses. Crimping cannot be explained by Farris banding[12] or lamina damage, and is most easily seen in fresh material.

We examined leaf crimping in detail in species of the perennial tussock grass *Chionochloa* (Arundineae), an ecologically widespread genus in New Zealand[13]. Crimping occurred in all 22 species. The degree of crimping differs between species of *Chionochloa*, and appears to be a function of the rate of leaf production and other factors such as ligule structure and lamina development. Primary crimping is caused when the most recently differentiated ligule presses directly on the soft tissue of an enclosed younger leaf. Other enclosed leaves are simultaneously but indirectly crimped, with progressively less distortion towards the center of the shoot (fig. 1 a). Primary crimping permanently constricts the leaf-blade, and

Table 1. Grasses showing primary crimping

Arundineae
Arundo donax L., *Chionochloa* Zotov (22 species), *Cortaderia fulvida* (Buchanan) Zotov, *C. selloana* (Schultes & Schultes f.) Asch. & Graebn., *Lamprothyrsus peruvianus* A. Hitchc., *Pyrrhanthera exigua* (Kirk) Zotov, *Rytidiosperma nudum* (Hook. f.) Connor & Edgar, *R. setifolium* (Hook. f.) Connor & Edgar, *R. thomsonii* (Buchanan) Connor & Edgar

Aveneae
Agrostis capillaris L., *Ammophila arenaria* (L.) Link, *Anthoxanthum odoratum* L., *Hierochloe redolens* (M. Vahl.) Roemer & Schultes

Bromeae
Bromus mollis L., *B. tectorum* L., *B. diandrus* Roth

Cynodonteae
Zoysia minima (Colenso) Zotov

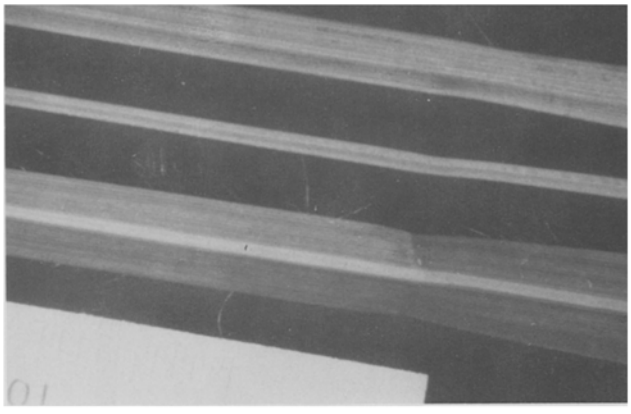
Eragrostideae
Distichlis distichophylla (Labill.) Fassett

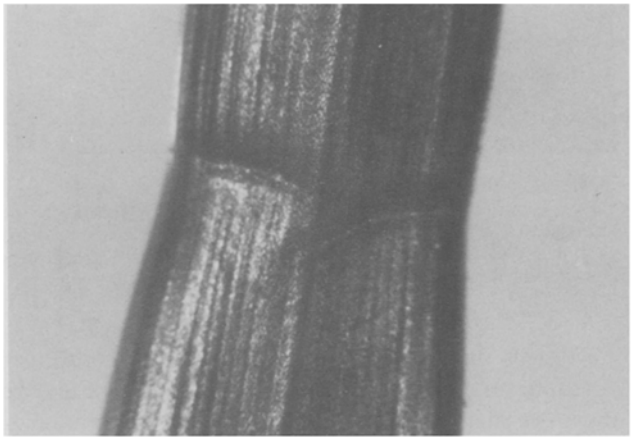
Ehrharteae
Microlaena avenacea (Raoul) Hook. f., *M. stipoides* (Labill.)R. Br.

Poeae
Dactylis glomerata L., *Festuca arundinacea* Schreb., *F. rubra* L., *Lolium perenne* L., *Poa annua* L., *P. cita* Edgar, *P. kirkii* Buchanan, *Vulpia bromoides* (L.) S. F. Gray

Stipeae
Anemanthele lessoniana (Steud.) Veldk., *Piptatherum miliaceum* (L.) Cosson

Triticae
Elymus enysii (Kirk) Löve & Connor, *Elytrigia repens* (L.) Nevski


a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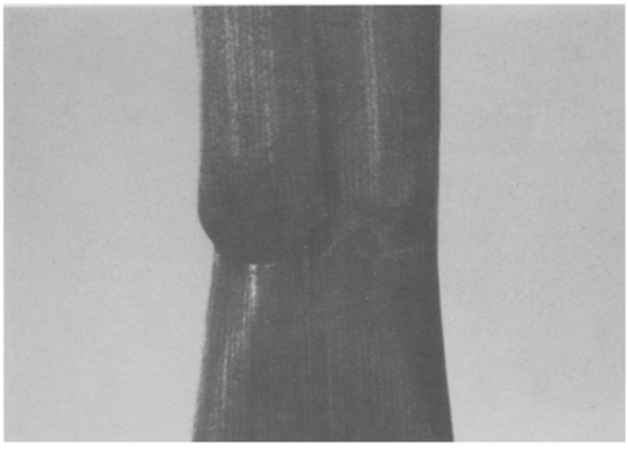
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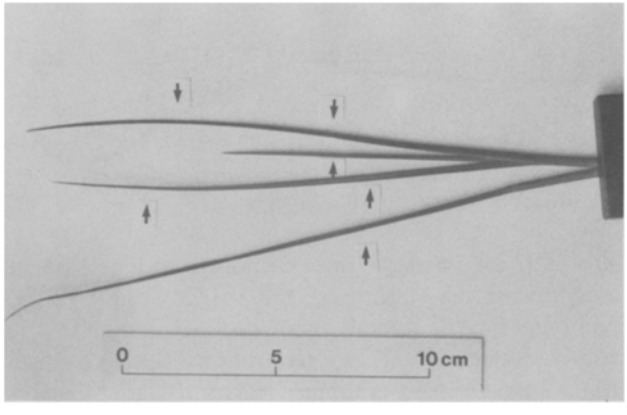

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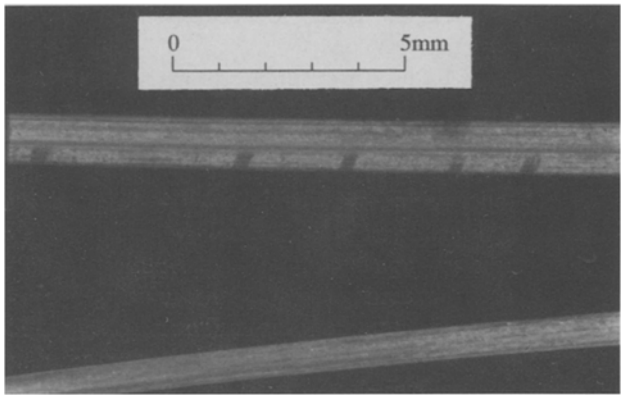

Figure 1. Primary and secondary leaf crimping on lamina of different grasses.
a Primary abaxial crimp on a leaf of *Chionochloa pallens* and synchronous indirect primary crimps on younger leaves (×10). *b* Primary crimping on abaxial lamina of *Chionochloa crassiuscula* (×10). *c* Primary crimping on abaxial lamina of *Festuca arundinacea* (×10). *d* Position of primary crimps in a shoot of *C. crassiuscula*. *e* Secondary crimping on abaxial lamina of *Chionochloa macra* showing variation in inter-crimp lengths similar to experimentally generated daily leaf growth rates (×10, see fig. 3).

in most species forms a clearly defined abaxial transverse groove on the blade (fig. 1 b, 1 c). Older ligules do not cause primary crimping because the tissue of the mature leaves they contact has hardened.

The number of crimps per leaf is inversely related to growth rate. Rapidly growing species have a single primary crimp per lamina, e.g., *Poa annua*. Slow-growing, perennial species may have several crimps per lamina, e.g., *Chionochloa pallens* Zotov has three or four. Each *C. pallens* leaf is crimped by three or four separate ligules before it is displaced from the centre of the shoot (table 2). Other species, e.g., *C. crassiuscula* (Kirk) Zotov have fewer crimps but the process is identical (fig. 2). The crimp nearest the tip is usually the least distinct, often merely a subtle change in lamina alignment, but the crimp nearest the sheath is always the most sharply defined, because the lamina was in direct contact with the ligule (fig. 1 a).

Secondary crimping is caused in some species by diurnal differences in the rate of leaf extension. At night, when there is minimal extension, the ligule of a fully expanded leaf constricts the enclosed growing leaf long enough to mark the abaxial surface (fig. 1 e). As the strengthening subepidermal sclerenchyma in the growing leaf are fully developed by this stage the crimps are less conspicuous than primary crimps, except on laminae coated with epicuticular wax. Daytime leaf extension is recorded by the distance to the next crimp, thus providing a continuous record of variation in daily growth rates (fig. 1 e). Secondary crimping may be transient (e.g. *Chionochloa macra* Zotov) or may persist on older leaves (e.g. *Cortaderia selloana*).

We grew *C. macra* tussocks in a controlled environment experiment to confirm the proposed mechanisms for primary and secondary crimping. We expected: (1) young leaves to grow at the same rate; (2) reduced leaf growth at night; (3) secondary inter-crimp distances to be correlated with rates of leaf extension.

Table 2. Lamina inter-crimp length (mm) in a *C. pallens* shoot

	Emerged leaves*				Emerging leaves	
	1	2	3	4	5	6
C3 to C4#	176	233	164	162		
C2 to C3	-	175	233	166	162	162
C1 to C2	-	-	175	233	166	166

* Leaves numbered from the outermost live leaf. # Primary crimps numbered by chronological formation; inter-crimp sections from the same crimping shown in similar typeface. - Lost by lamina breakage.

Leaves on two or three mature primary shoots in six tussocks from montane and alpine populations, pregrown in a glasshouse for 6 months, were cut level with an adjacent dead leaf after 2 days acclimatisation in a controlled environment cabinet. Leaf length (±1 mm) was measured after 7 days at montane mid-summer conditions[14] (14-h day at 20 ± 2 °C, light intensity 850–900 μ E at canopy level, relative humidity 70 ± 5%; 8-h night at 10 ± 2 °C, and 85% ± 5% relative humidity; 2-h intermediate temperatures and relative humidities, 90–100 μ E light intensity) and a further 7 days at late autumn temperatures[14] (day lengths and light intensity as previously, day temperature 10 ± 2 °C, relative humidity 90 ± 2%; night temperature 2.5 ± 1 °C, relative humidity 95 ± 2%).

Fifteen pairs of innermost leaves were examined for elongation rate. Nine pairs showed no difference, four pairs differed by 1 mm, one by 2 mm and one pair by > 3 mm (Chi-square = 11.4, $p < 0.01$). Since experimental accuracy was ±1 mm this result establishes that young pairs of innermost leaves are extending at the same rate, confirming the described mechanisms for primary crimping. Leaf extension slowed at night, providing sufficient time for secondary crimping. Daily leaf growth rates were directly related to the experimental environmental conditions (fig. 3) and were consistent with field[15,16] and oth-

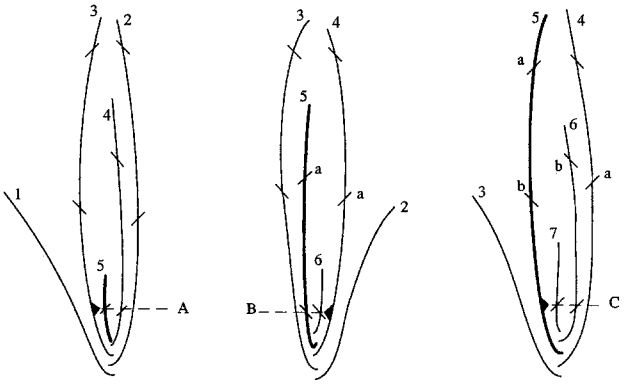

Figure 2. Position and sequential formation of primary crimps on a shoot of *Chionochloa crassiuscula* (Cf. fig. 1 d). Synchronous crimps are indicated by common letter. Leaf numbering as in table 2. (a) Leaf 3 ligule crimps leaves 4 and 5 at 'A'; (b) Leaf 4 ligule crimps leaves 5 and 6 at 'B'; (c) Leaf 5 ligule crimps leaves 6 and 7 at 'C'.

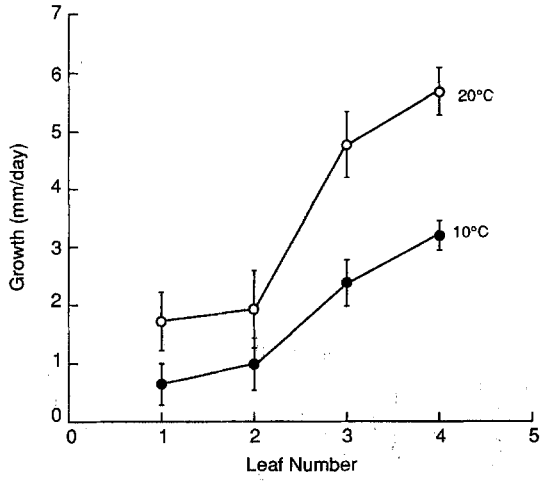

Figure 3. *Chionochloa macra* lamina daily growth rate at 20/10 °C and 10/2.5 °C day/night temperature environments (mean ± 95% Confidence Interval). Growth rates differed significantly ($p < 0.0003$, ANOVA).

er experimental data[17]. Leaf extension was similar in magnitude to observed secondary inter-crimp lengths (fig. 1e). This supports the use of secondary crimping as a naturally occurring record of daily growth rate in grasses.

Crimping provides an intrinsic history of shoot growth. Secondary crimping gives a naturally occurring record of daily growth rate in grasses. This makes available a new tool for investigating the relationship between plant performance and environmental factors such as weather, without prolonged field observation. Crimping can be used to accurately determine relative tissue age in a shoot. The distance between primary crimps (table 2; fig. 2) shows the synchronism. Precise location of similar aged tissue will be useful for ecophysiological and metabolic studies.

Crimping can be used to estimate primary production through leaf-blade length-weight ratios[16] or as a direct measure by destructive sampling. It can also be used to compare growth between plants and populations.

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Host trail following by the guest ant *Formicoxenus provancheri*

A. Lenoir[a], C. Detrain[b] and N. Barbazanges[a]

[a] *Laboratoire d'Ethologie et Sociobiologie, URA CNRS 667, Université Paris Nord, Av. J. B. Clément, F-93430 Villetaneuse (France), and* [b] *Laboratoire de Biologie Animale et Cellulaire, Université Libre de Bruxelles, Av. F. D. Roosevelt 50, B-1050 Bruxelles (Belgique)*

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Abstract. Formicoxenus provancheri, a guest ant of *Myrmica incompleta*, is able to follow artificial trails made with the poison gland secretion of its host. The trail-following response is elicited at the same range of concentrations as for the host species. The performance of *Formicoxenus* is enhanced by the presence of the host. The adaptive value of these phenomena is discussed.

Key words. Artificial trails; trail-following; guest ants; parasite dissemination; Myrmica; *Formicoxenus*.