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Morphological Constraints of Shoot Demography of a Clonal Plant: Extra- and Intravaginal Tillers of *Festuca rubra*

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Abstract ***Festuca rubra*** **forms tillers in two different ways: extravaginally and intravaginally. Demography of these two tiller types was observed in seventeen selected tussocks of *Festuca rubra* s.s. over four growing seasons. Extravaginal tillers were bigger at birth and on the average produced twice as many daughter tillers per tiller. In general, the natality and mortality of extravaginal tillers were less regular than that of intravaginal tillers. Overall tillering rate per tiller was correlated with the density of the surrounding vegetation; mortality, natality and tiller life span were not. High density of the surrounding vegetation did not result in increased formation of extravaginal tillers. The proportion of the extravaginal tillers was not correlated with the density of the *F. rubra* tussocks. There is no evidence for foraging by extravaginal tillers, but they do act as founders of small clusters of tillers.**

Key words: **branching, daughter shoots, grasslands, survival rate.**

In plants with complex branching systems, there is an intimate relation between the module demography and morphology/branching system (White, 1979; Waller and Steingraeber, 1985). In grasses the daughter tillers are formed in two different ways, viz. within the sheath of the mother tiller (intravaginally) and outside the sheath (extravaginally) (Fig. 1). These two branching modes have important consequences for tussock architecture (Stace et al., 1992) and for the ability of the particular grass species to form mixed swards with other species. Prevalence of intravaginal tillers leads to the formation of dense tussocks, which may behave as phalanx strategists within a community; extravaginal tillers represent a more guerrilla type of behaviour, especially if extravaginally formed branches develop into rhizomes bearing tillers.

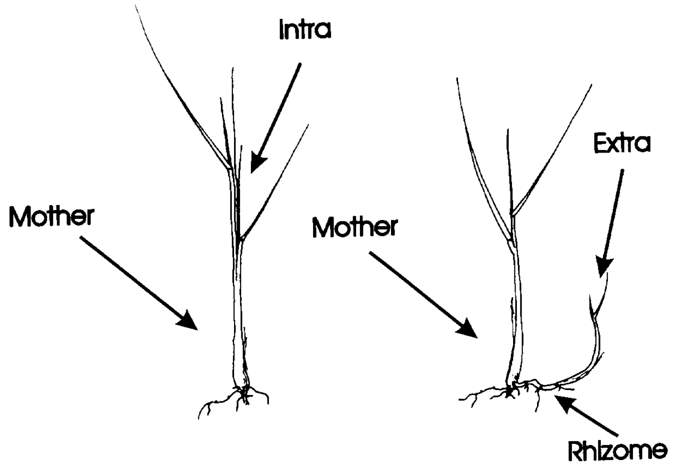

Fig. 1. Morphology of intravaginal and extravaginal tillers of *Festuca rubra*. Mother—mother tiller, intra—intravaginal daughter tiller, extra—extravaginal daughter tiller.

In some grasses, only one mode of tiller formation is present; other species are able to shift from one mode to the other. Species able to form both tiller types may be able to exploit their environment by changing the proportion of extravaginal tillers. This would constitute a clear morphogenetic means of foraging for space (de Kroon and Knops, 1990), but no data are available to demonstrate its existence.

Surprisingly, effects of the branching system on the demography of grasses have not been systematically explored, in spite of widely acknowledged importance of demographical processes for grass performance (Danais, 1984; Sydes, 1984; Garnier and Roy, 1988; Kotanen and Jefferies, 1987; Mitchley, 1988; Butler and Briske, 1988; Bazely and Jefferies, 1989; Jónsdóttir, 1991). The present study hence attempts to address the following problems:

—how do extravaginal and intravaginal tillers differ in their demography and number of daughter tillers within a community, and,

—is there any relation between the proportion of extravaginal and intravaginal tillers and the density of the surrounding vegetation.

As a model species we used *Festuca rubra*, which is able to form both types of tillers and in which extravaginal tiller formation is at least partly under environmental control (Rytova, 1971; Krahulec et al., unpubl. data). The demography of both tiller types and its effects on the tussock growth are studied in a traditionally managed mountain meadow.

Material and Methods

1. *Study Site*

The data were collected in a mountain grassland in the Krkonoše Mts., NW part of the Czech Republic (Severka settlement, ca. 3 km NW of Pec pod Sněžkou, altitude approx. 1100 m above sea level). The growing season starts in about mid May when snow melts away (e.g. 10th May in 1988). Mean temperature in the warmest month (July) was 13.6°C (1988), 13.7°C (1989). The last month with average temperature above zero was October. The grassland under study is managed in the traditional way, i.e. mowed once or twice a year (depending on the weather) and grazed late in the autumn. It was manured once in several years. The grassland is rather species poor, with only five important species: *Anthoxanthum alpinum, Deschampsia flexuosa, Festuca rubra, Nardus stricta* and *Polygonum bistorta*. These species always accounted together for 95% of the total living aboveground biomass. From the phytosociological point of view, the grassland can be classified into Sileno-Nardetum pleurozietosum (Nardo-Agrostion alliance, Nardetalia order, see Krahulec, 1990). *Festuca rubra* occurs here in loose tussocks partly mixed with other grasses.

The small scale community structure of the grassland is rather complex. In a 3×3 cm square, there are 1 to 6 species (average 2.1–2.8 depending on the site). The density of *F. rubra* tillers in the *F. rubra* occupied squares (3×3 cm in size) ranges from 1 to 20; averages for tussocks range from 3.1 to 5.6 (Krahulec et al., unpubl. data). All *F. rubra* belongs to the hexaploid *F. rubra* s.str., which is capable to form both intra- and extravaginal shoots.

2. *The Species*

F. rubra in the widest sense is known to consist of a hierarchy of types (microspecies, subspecies) with different degrees of ecological specialization and ploidy (Markgraf-Dannenberg, 1980). The formation of extravaginal tillers and rhizomes and the extent to which this process is environmentally controlled are some of the principal characters distinguishing these types. In more plastic types, such as hexaploid *F. rubra* s.s., the formation of extravaginal tillers is variable and dependent on the environment (Krahulec et al., unpubl. data). Though there is an ample knowledge of the morphogenetic processes of both intra- and extravaginal tiller formation (Rytova, 1972), the factors involved in their regulation in the field are still not well understood.

3. *Data Collection*

Seventeen tussocks of *F. rubra* were selected for the study. All of them were located in an area of ca. 12×3 m. The tussocks were selected with *a priori* requirements that (i) there be no *F. rubra* within a 10 cm radius to prevent uncertainty in delimiting the tussocks in further measurements, and (ii) they have a workable number of tillers (less than 25 at the beginning).

All tillers within tussocks were marked throughout the study. Tillers were marked using coloured plastic rings (2–4 mm in diameter and 3–5 mm in length) marked with 0–4 notches (to increase the number of combinations). These rings were split on one side so that they could be opened and easily put on grass tillers from the side; the material was elastic enough to hold the ring on the tiller over the whole observation period. The ring was put on the tiller below the insertion of the lowest leaf blade.

At each recording, the number of leaves and the length of the longest leaf blade were recorded for each tiller. These two variables account for 64% of the total variation in the tiller mass (Herben et al., 1993). All green leaves were counted; partially green leaves were counted if the green part of the blade exceeded 10 mm; young developing leaves were counted if the length of the exserted part exceeded 10 mm. Altogether 1251 tillers were observed.

Additional information was collected for newly appeared tillers. Their origin, whether intravaginal and extravaginal, was recorded. All tillers developing under the leaf sheath of a mature tiller and more or less parallel with it were considered to be intravaginal; since seedlings were not observed (unpubl. data), all tillers appearing in the sward without any obvious connection to already established tillers were considered extravaginal. This distinction was not difficult to make, since the sheaths of leaves of mother tillers within which the daughter tillers developed were generally well preserved during the short recording intervals. The identity of the mother tillers of intravaginal tillers was recorded.

Observations started in the spring 1987 and continued until the summer 1990. Three censuses were made before the clipping and two to four recordings afterwards. The first spring census was done approximately ten to fourteen days after the snow had melted away; the last autumn census was done as late as possible before the permanent snow cover. Exact dates of the recordings were: in 1987, 4/6, 25/6, 14/7, 5/8, 29/8, 14/9, 10/11; in 1988, 24/5, 23/6, 9/7, 3/8, 5/9, 12/10; in 1989, 19/5, 15/6, 8/7, 7/9, 4/10; in 1990, 10/5, 15/6, 10/7.

Every year in mid July the tussocks were clipped together with the vegetation surrounding them, to the height of 2.5 cm, to simulate the traditional mowing of the grasslands.

In July 1987, the vegetation immediately surrounding the tussocks was recorded. The recording was made using a 3.3×3.3 cm grid established over each

Table 1. Differences between tillers formed extravaginally and intravaginally. For size difference see Fig. 3.

	Intravaginal	Extravaginal	Test	Statistic	Signif.
Number of tillers	866	175			
Mean life span (days)	544[1]	623[1]	Mantel-Cox	5.41	0.020
Percentage of flowering tillers	17.6	20.8	chi-square	2.06	n.s.
Number of daughter tillers per lifetime	0.39	0.77	Mann-Whitney	5.92	0.0001

[1] estimated using the product moment (Kaplan-Meier) estimate.

tussock. The grid was large enough so that each tussock was surrounded by at least one row of 3.3 × 3.3 cm cells containing no *F. rubra* tillers. The number of tillers of all grasses within each cell was counted. These data were used to express (i) mean density of *F. rubra* within cells covering the tussock, (ii) mean density of all grasses in these cells , (iii) mean density of all grasses in the tussock neighbourhood (i.e. in the cells either covering the tussock or surrounding it).

Standardized size of tillers was calculated as a mean size (either number of leaves or length of the longest leaf) divided by the mean for all sterile tillers of that recording time. Natality and mortality was expressed as number of newly formed tillers per tiller population size per unit time. Survivor functions were estimated using the product limit (Kaplan-Meier, see Dixon, 1991) method. Differences in survival times between the two tiller types were tested using the Mantel-Cox statistic (Dixon, 1991).

Results

1. *Demography of Intravaginal and Extravaginal Tillers*

Most newly formed tillers were formed intravaginally (866 out of 1041, 83%). Extravaginal tillers differed from intravaginal tillers by many of their life history characteristics (Table 1). The average life span of extravaginal tillers is slightly longer than that of intravaginal tillers; however, the survival of older extravaginal tillers is considerably longer than the survival of older intavaginal tillers (Fig. 2). The extravaginally formed tillers were on average bigger at the time of their birth; the difference is significant both for number of leaves and length of the longest leaf (Fig. 3.). Tillers formed extravaginally produced almost twice as many daughter tillers during their lives. In contrast, the rate of flowering does not significantly differ between extravaginal and intravaginal tillers.

The seasonal pattern of natality of extravaginal tillers is similar to that of intravaginal tillers, with a maximum

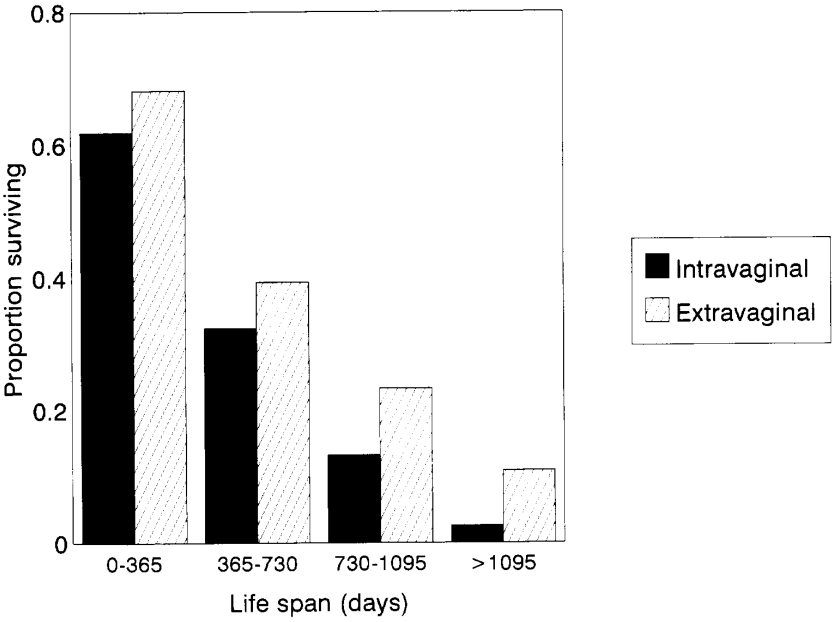

Fig. 2. Survivorship of intravaginal and extravaginal tillers.

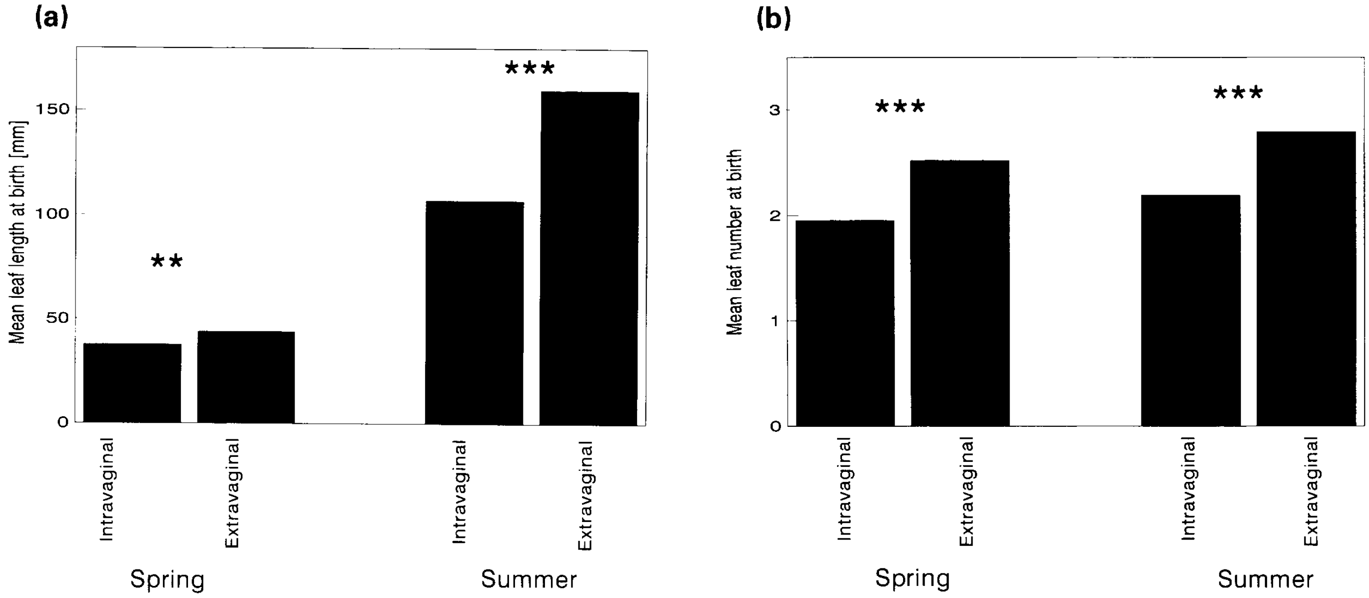

Fig. 3. Differences in size measured as (a) leaf length and (b) number of leaves at birth between extravaginal and intravaginal tillers. Spring—tillers born during winter and early spring (recorded as new in the first spring recording), Summer—tillers born in early summer before mowing. Difference between intravaginal and extravaginal tillers is significant using one way ANOVA (**, $P<0.01$; ***, $P<0.001$).

in the late spring (Fig. 4). There are two main differences in this pattern between intravaginal and extravaginal tillers: the proportion of extravaginal tillers is slightly greater in late summer and autumn (i.e. the spring peak of natality is weaker); furthermore, the time courses of natality of both tiller types are different from one another during the year 1989. The difference in the time course of natality of extravaginal vs. intravaginal tillers is significant (Kolmogorov-Smirnov two sample test, DN=0.6, $P<0.01$).

The proportion of intravaginal vs. extravaginal tillers varied between individual tussocks; intravaginal tillers accounted from 4 to 38% of the total newly formed tillers during the observation period.

2. *Effects of Surrounding Vegetation*

The overall grass density around the *F. rubra* tussocks varied from 5.4 to 27.2 tillers/3×3 cm square. The most abundant species occurring together with *F. rubra* at the level of the 3×3 cm squares was *Deschampsia*

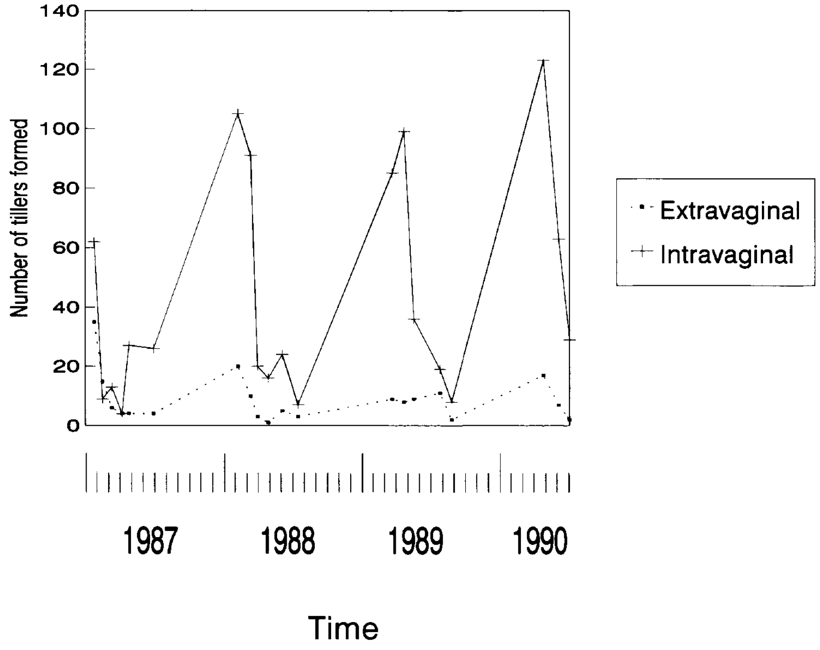

Fig. 4. Time course of the relative natality of intravaginal (full line) and extravaginal (dashed line) tillers. Natality and mortality are standardized per tiller and time period.

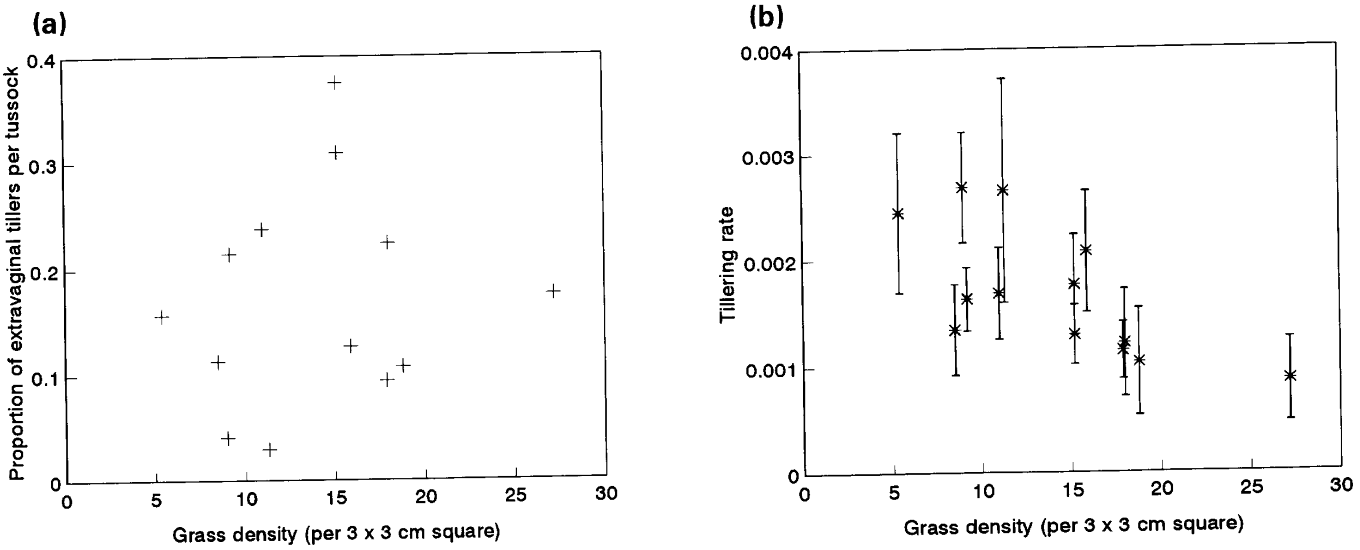

Fig. 5. The relation of the overall density of grass tillers with (a) the proportion of extravaginal tillers (Spearman's $R=0.091$, $P=0.753$) and (b) the overall tillering rate (expressed as mean number of daughter tillers per tiller divided by the life span of the tiller; Spearman's $R=-0.710$, $P=0.014$). Points represent individual tussocks, bars indicate standard deviations. Only tussocks with more than the average 10 tillers during the observation are included.

flexuosa, whose density varied from 1.7 to 20.6 tillers/3×3 cm square. The qualitative and quantitative species composition of surrounding squares did not differ from the squares with studied *F. rubra* tillers except for the absence of *F. rubra* from the surrounding squares.

The tillering rate of tussocks (expressed as mean number of daughter tillers per tiller per year) was negatively correlated with overall grass density (Spearman $R=-0.71$, $P=0.014$, Fig. 5b), but not significantly correlated with the *F. rubra* density ($R=-0.47$, $P=0.105$). The proportion of extravaginal tillers in the tussock was not correlated with the mean grass density around the tussock (Spearman's $R=0.066$, $P=0.79$, Fig. 5a).

The density of *F. rubra* (i.e. the compactness of the *F. rubra* tussock) ranged from 1.2 to 9.0 tillers per 3×3 cm square. It was not correlated with the proportion of extravaginal tillers (Spearman's $R=0.076$, $P=0.76$).

Discussion

1. *Tussock Structure and Extravaginal vs. Intravaginal Tiller Formation*

The extravaginal tillers differ from the intravaginal tillers in several respects; they are generally more vigorous than tillers formed intravaginally. Since they produce more daughter tillers and live longer, they serve as founders of small clusters of tillers. In this respect they do seem to have a different role from intravaginal tillers. Because of these differences, the proportion of extravaginal tillers within a tussocks puts a constraint on the tiller demography of that tussock.

On the other hand, nothing in the present study indicates the extravaginal tillers behave as foragers for free space. There is a large variation in the surrounding vegetation density in the study grassland (spanning almost one order of magnitude); it has a strong effect on the overall tillering rate within the tussocks (Fig. 5). In such conditions, a species with environmentally controlled formation of spacers (i.e. extravaginal tillers) should increase their formation in denser vegetation. In contrast to this expectation, there is no relationship between the proportion of extravaginal tillers in a tussock and the vegetation density. However, it remains unclear how the proportion of extravaginal tillers would change in stands with experimentally manipulated density, especially at much lower densities than in this study.

The large variation in proportion of extravaginal tillers could have two (not necessarily exclusive) explanations. (i) The measure of density employed here may not be adequate to describe the environments of individual tillers within a tussock. The 3×3 cm scale may be too coarse to detect within tussocks regulation. In addition, calculating the averages for tussocks may smooth out the data. (ii) There may be a genetic component to the differences in extravaginal tiller formation between individual tussocks. This question cannot be solved using the present data set. From the taxonomic point of view, all tussocks belong to the same taxon; however, given the large variation between clones within one grassland (Skálová and Krahulec, 1992), the genetic differences between tussocks cannot be ruled out. Preliminary data indicate that each tussock belongs to a separate genotype except tussock 2 and 3

(Suzuki et al., unpubl. data).

The compactness of the tussocks of *F. rubra* is often supposed to be due to the proportion of extravaginal tillers and rhizomes. Culture experiments comparing several taxa with contrasting morphology showed that the proportion of extravaginal tillers indeed is a crucial factor in density determination (Krahulec et al., unpubl. data). Clones with a high proportion of extravaginal tillers generally have low final tiller density in the tussock. In our study, however, the compactness of the tussock (i.e. the mean density of *F. rubra* tillers) is not related to the proportion of extravaginal and intravaginal tillers in spite of the large variation in this parameter. The absence of correlation shows that the spatial structure of the vegetation is not related to the demographic processes within the tussock.

The potential number of daughter tillers is limited by the number of buds, which is equal to the number of formed leaves. In the studied grassland, the average tiller forms approximately 3.5–4.5 leaves per year (Hájek, 1989); since fewer than one intravaginal daughter tiller is produced per tiller per year (0.586), the ratio of developing to dormant buds is about 0.1 to 0.2. This indicates a strong suppression of tiller formation in the grassland, which makes most of the buds dormant and available for later formation of extravaginal tillers and/or regeneration.

In addition to their morphological differences, extravaginal and intravaginal tillers also differ in the timing of their development. The spring peak of intravaginal tiller formation corresponds to the period of intense growth in spring and is a well known phenomenon (Rabotnov, 1985; Colvill and Marshall, 1984; Butler and Briske, 1988). Whereas intravaginal tillers develop sylleptically (i.e. at the same time as the growing terminal bud), the extravaginal tillers develop from dormant buds after a considerable delay (Rytova, 1971). The lower regularity of extravaginal tiller formation observed in the present study may indicate that the activation of these dormant buds responds to different and more variable stimuli than the formation of sylleptic intravaginal tillers. This is supported by other observations available (Serebrjakova, 1973; Hájek, 1989).

Conclusions

Intravaginal and extravaginal tillers do behave differently in the field grown tussocks of *F. rubra*. If the functional differences between tillers of different origin are more widespread in other grass species, this would constitute an important component of the regulation of the grass growth form and its growth dynamics.

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