Effect of Vernalisation and Tiller Age on Seed Production in Tall Fescue.

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ABSTRACT

A vernalisation study showed that a period of 1440 hours (60 days) of vernalisation was necessary to vernalise all plants of tall fescue (*Festuca arundinacea* Schreb.) cv. Grasslands Roa. Plants receiving no vernalisation did not produce seed heads. The vernalisation study also confirmed that a juvenile stage does not exist in Roa tall fescue. A field study showed that over 90% of the seed heads present at anthesis were from tillers produced the previous late–summer and autumn. Management of seed crops in relation to length of vernalisation and tiller age is discussed.

***Additional index words*: reproductive tillers, juvenility.**

INTRODUCTION

A recent study (Hare, 1993a) found that after 960 hours (40 days) of vernalisation, 36% of tall fescue (*Festuca arundinacea* Schreb.) cv. Grasslands Roa plants failed to produce seed heads. It was suggested that these plants had not received a sufficiently long vernalisation period and a further study, subjecting tall fescue plants to longer vernalisation periods was recommended. Additionally this would determine whether a juvenile stage is present in tall fescue. Hare (1993a) in part discounted the juvenile stage suggested by Bean (1970), but because 36% of the plants remained vegetative after 960 hours of vernalisation, the juvenile stage could not be completely dismissed.

Hare (1993a) found that 10% of unvernalised Grasslands Roa tall fescue plants produced seed heads. This was a lower proportion than the 44% of plants of a French tall fescue cultivar that produced seed heads when kept continuously at temperatures above 15°C (Hicks and Mitchell, 1968). Hare (1993a) suggested that the glasshouse used for flowering in his study may have allowed enough short day length cycles to induce flowering in the control plants. Low temperatures alone may not be an absolute requirement for induction of flowering in tall fescue (Hicks and Mitchell, 1968). Further work was therefore required to show whether Roa tall fescue required vernalisation to flower and whether juvenility restricted flowering in tall fescue.

A knowledge of the number of tall fescue tillers that originate at different times of the year, what proportion become reproductive, and how much they individually contribute to seed yield would be useful in order to manipulate field crop management for seed production. If tall fescue requires a period of vernalisation greater than 40 days for maximum seed yield, then it is likely that the majority of seed heads present at harvest will be produced by tillers initiating in the previous late summer and autumn, as found in field studies with other perennial grass species (Langer and Lambert, 1959; Ryle, 1964; Hill and Watkin, 1975). In pot trials with tall fescue Robson (1968) found that summer and early autumn–formed tillers contributed most to seed yield and that late autumn–, winter– and spring–formed tillers lived only a short time and did not become reproductive. However, no field studies of tall fescue tiller formation under standard seed crop management have been published. By knowing when the most productive reproductive tillers first originate in the field and their length of vernalisation, seed growers could adjust autumn sowing in first year crops and time post– harvest management and autumn–winter defoliation practices in older crops to encourage maximum production of reproductive tillers.

Because of this lack of precise information on vernalisation requirements and the need for a better understanding of reproductive tiller formation and contribution to seed yield, it was decided to compare the effects of different vernalisation periods on reproductive tiller production under controlled growth–room conditions, and to measure the contribution of the individual tillers emerging at different times to seed production in the field.

MATERIALS AND METHODS

Controlled vernalisation

Seeds of tall fescue cv. Grasslands Roa were germinated on moist filter paper at 25°C and four days later, on 21 June 1993, two sprouted seeds were sown per 15 cm diameter pot. Pots contained a potting mixture previously described by Hare (1993a). The pots were immediately placed in a controlled warm environment room (18°C, 16 hour daylength) and 14 days after sowing, plants were hand–thinned to one plant per pot.

Twenty eight days after sowing, when the plants had 1–2 leaves unfolded on the main shoot and 3–5 tillers (Decimal code 23 & 25 in Zadoks, Chang and Konzark 1974 and Tottman and Broad 1987), they were transferred to the vernalisation room for 20, 40, 60 or 80 days. A set of unvernalised plants remained in the controlled warm environment room. The daylength of the vernalisation room was 8 hours with day/night temperatures of 8/4°C. Further conditions of the vernalisation room are detailed in Hare (1993a). According to Langer (1972) the most effective vernalising temperatures lie between 0 and 10°C and as the extent of the response can vary, it was decided that the maximum and minimum thresholds for vernalisation in this controlled study would be 8 and 4°C respectively. In order to avoid the possibility of any devernalisation, the maximum threshold of 8°C was

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chosen so as to be well within the effective vernalisation range. The 24 plants (one plant per pot) in each treatment were divided into 4 replicates and randomly placed in each treatment block. Plants in the five treatments (0, 20, 40, 60 and 80 days vernalisation) totalled 120 plants.

Following their respective vernalisation treatments the plants were then transferred back to the controlled warm environment room (18°C, 16 hour daylengths) with the unvernalised plants until flowering.

Beginning at seedling emergence all tillers on the plants that were to be vernalised were tagged as they emerged from the leaf sheath using different coloured plastic rings. Tillers were tagged prior to and during vernalisation, but tagging was discontinued following vernalisation. All tillers that had emerged and were exposed to vernalisation conditions were therefore identified. After returning to the warm environment room, vernalised and unvernalised plants were grown through until tillers reached anthesis. At their time of anthesis individual reproductive tillers on each plant were cut and the number of spikelets per tiller and florets per spikelet determined (one spikelet at the base, middle and top of each tiller was counted to estimate floret numbers).

Origin of reproductive tillers in the field

Nine plots each of tall fescue cv Grasslands Roa and cv Grasslands Advance were established at the AgResearch Grasslands farm, Manawatu (40° south), in autumn 1991, and harvested for seed in December 1991 and December 1992. Details of crop management, plant development and seed yields are detailed in Hare (1994).

In January 1993 the straw was removed by a forage harvester and the stubble hard grazed to ground level by sheep. The plots were again hard grazed by sheep to 3 cm above ground level on 12 February and lightly grazed by sheep to 5 – 7 cm above ground level on 19 March. Nitrogen as urea was applied on 23 April (50 kg N ha^{-1}) and 1 September 1993 (100 kg N ha^{-1}). Atrazine (2 kg ai ha^{-1}) and MCPA (1.5 kg ai ha^{-1}) were applied on 23 April 1993.

In each of the 18 plots (9 of Roa and 9 of Advance) one 25 x 25 cm area of tall fescue was permanently marked. All newly emerged tillers within this 25 x 25 cm area were tagged with different coloured plastic rings on the 22nd of each month, beginning in February and ending in September. At each tagging the number of new tillers and the number of dead tillers were recorded. During anthesis (29 November 1993) tagged tiller areas were dug out and the tillers examined in the laboratory. All tillers were classified into 'reproductive', 'vegetative' and 'dead' categories, according to their month of tiller origin. Reproductive tillers were analysed for spikelet and floret numbers.

During the course of the field study hourly grass minimum temperatures, 10 cm soil temperatures, air temperatures and rainfall were recorded at a nearby meteorological station.

RESULTS

Controlled vernalisation

All plants produced new tillers during the course of the vernalisation period. On average 2, 4, 8 and 13 new tillers per plant were produced following 20, 40, 60 and 80 days vernalisation, respectively.

All plants vernalised for 60 and 80 days produced reproductve tillers (Table 1) whereas only 83% of plants vernalised for 40 days produced reproductive tillers and only 17% of plants vernalised for 20 days produced reproductive tillers. None of the control plants (0 days vernalisation) produced reproductive tillers (Table 1). All the vegetative tillers that were tagged before the 60 and 80 day vernalisation treatment became reproductive (Table 2).

Plants vernalised for 60 and 80 days produced twice the number of reproductive tillers per plant but fewer spikelets per tiller than plants vernalised for shorter periods of time (Table 1). Plants vernalised for 80 days produced more florets per spikelet than plants from other treatments.

Table 1. Reproductive development of plants vernalised for various times in a controlled environment room.

Days of vernalisation	% of plants with reproductive tillers[1]	No of reproductive tillers per plant[1]	Spikelets per reproductive tiller	Florets per spikelet
0	0	0	0	0
20	17	8(1.9)[2]	86(7.6)	5.8(0.2)
40	83	8(1.0)	91(4.4)	5.2(0.1)
60	100	14(1.1)	73(1.9)	5.4(0.1)
80	100	18(1.1)	60(1.8)	6.4(0.1)

1 *from 24 plants per treatment*
2 *Standard error of mean*

Table 2. The percentage of vegetative tillers which emerged before and during vernalisation in a controlled environment room that became reproductive.

Days of vernalisation treatment	% of vegetative tillers that became reproductive	
	emerged before vernalisation	emerged during vernalisation
20	13	12
40	62	42
60	100	71
80	100	87

Many of the tillers that became reproductive had not emerged before the vernalisation period began (Table 3). The longer the period of vernalisation, a greater number of reproductive tillers were formed from vegetative tillers that emerged during the vernalisation period (Table 3). Plants that received 80 days vernalisation produced more than twice the number of reproductive tillers from vegetative tillers that emerged during vernalisation than from vegetative tillers formed before vernalisation (Table 3).

Origin of reproductive tillers in the field

At anthesis 94% of the Roa reproductive tillers and 92% of the Advance reproductive tillers present had originated from vegetative tillers formed before the end of April (Table 4). Virtually all the tillers produced from June to September were still vegetative at anthesis.

Table 3. The percentage of reproductive tillers that arose from vegetative tillers emerging before, during and after vernalisation in a controlled environment room.

Days of vernalisation	% of reproductive tillers formed from vegetative tillers that:			Total no. of reproductive tillers
	emerged before vernalisation	emerged during vernalisation	emerged after vernalisation	
20	50	17	33	30(4 plants)
40	52	28	20	144(20plants)
60	35	40	25	334(24plants)
80	25	60	15	416(24plants)

During the autumn and winter (March – July), tillers died regularly at a rate of between 1.4 and 5% per month, except for Advance tillers formed in May of which over 6% died in June (Table 5). Over 6% of Roa tillers formed in May died in August alone. The greatest period of tiller death was from the end of September to anthesis (end of November), when between 30% to 60% of tillers present at the end of September died (Table 5). On average well over half the tillers formed had died by the end of November. Exceptions were Roa tillers formed in August and September where only approximately 35% had died by anthesis.

Rainfall was well distributed throughout the year and the mild temperatures (Table 6) enabled tall fescue to continue growing during the autumn and winter. By the end of September, tillers formed in February had received 2000 hours of temperatures below 8°C (Table 6). Tillers formed in May had received 1600 hours of temperatures below 8°C by the same date.

Table 4. Month of origin of tillers at anthesis in field plots

Cultivar	Month of Origin								Total
	February	March	April	May	June	July	August	September	
Roa									
No. of reproductive tillers[1]	188	44	17	10	2	3	0	0	264
No. of vegetative tillers[1]	238	89	75	61	89	103	92	61	808
No. of dead tillers[1]	443	198	183	176	126	146	56	31	1359
Total	869	331	275	247	217	252	148	92	2431
Advance									
No. of reproductive tillers[1]	217	26	30	17	3	3	2	0	298
No. of vegetative tillers[1]	284	37	92	87	88	107	60	21	776
No. of dead tillers[1]	459	204	175	188	155	138	80	26	1425
TOTAL	960	267	297	292	246	248	142	47	2499

[1] *Total No.from 9 x 0.06m² plots for each cultivar.*

Table 5. Percentage of tillers that died each month in relation to the month of origin.

Month of Origin	Month when tillers died								Total percentage of tillers that died
	March	April	May	June	July	August	September	October to anthesis	
Roa									
February	2.7	1.4	2.3	3.1	4.1	3.0	4.1	30.3	51.0
March	–	2.1	3.0	3.6	3.3	2.1	3.0	42.6	59.7
April		–	4.7	2.2	2.2	2.2	3.3	52.0	66.6
May			–	2.4	0.8	6.1	1.6	60.3	71.2
June				–	0.9	1.8	1.8	53.5	58.0
July					–	0.4	0.8	56.7	57.9
August						–	1.4	36.5	37.9
September							–	33.7	33.7
Advance									
February	3.1	1.8	2.2	4.4	4.0	2.0	2.3	28.2	48.0
March	–	1.5	1.9	3.7	2.6	4.9	1.9	59.9	76.4
April		–	2.4	2.7	3.7	3.7	2.4	44.1	59.0
May			–	6.2	1.0	1.7	4.8	50.7	64.4
June				–	0.8	2.4	1.6	58.1	62.9
July					–	0.4	1.6	53.6	55.6
August						–	2.1	54.2	56.3
September							–	55.3	55.3

Table 6. Average monthly rainfall, mean 10 cm soil temperature (0900 hours), mean grass minimum temperature (0900 hours), mean air temperature (0900 hours) and number of hours the grass minimum temperature was below 8°C for the field study trial site in 1993.

	Rainfall (mm)	Soil temperature (°C)	Air temperature (°C)	Grass minimum temmperature (°C)	No. of hours below 8°C
January	53.9	16.4	15.7	9.7	28
February	43.7	16.3	16.3	9.6	89
March	79.8	14.6	14.7	7.8	111
April	53.7	13.3	12.6	5.9	191
May	78.4	10.4	11.4	4.6	247
June	102.6	9.3	10.5	4.8	259
July	11.5	7.1	8.5	2.2	408
August	63.1	7.6	9.5	1.5	405
September	60.3	9.3	9.5	4.1	290
October	60.0	12.4	12.9	6.2	155
November	138.2	13.7	12.3	5.1	147

DISCUSSION

A previous vernalisation study (Hare, 1993a) did not clearly establish whether a juvenile stage exists in Roa tall fescue. Plants could be vernalised from any growth stage from main shoot and one leaf appearance onwards, thus in part discounting the prescence of a juvenile stage in tall fescue (Bean, 1970). However, because 36% of plants failed to produce seed heads after 40 days (960 hours) of vernalisation and remained vegetative, the juvenile stage could not be completely discounted. This study now shows that no juvenile stage exists in Roa tall fescue because after 60 days (1440 hours) of vernalisation, all plants produced seed heads. It is therefore the length of vernalisation and the number of tillers each plant has available to be vernalised that are of importance for maximum reproductive development in tall fescue. This study shows a quantitative vernalisation response because the longer the tall fescue plants were vernalised the greater the number of reproductive tillers formed.

Hare (1993a) suggested that the glasshouse conditions used in that study were marginal, as 10% of the unvernalised tall fescue plants produced seed heads. In the present study where unvernalised plants were grown at 18°C constant temperature with 16 hours of daylight, none of the unvernalised plants produced seed heads. The divergence in vernalisation requirements for tall fescue is apparently not as great as previously thought. If Hare (1993a) had used a controlled warm environment room for the unvernalised plants instead of a glasshouse, it is unlikely that any of the unvernalised plants would have become reproductive.

However, a small number (17%) of plants only required a minimum amount of vernalisation to produce seed heads (20 days, 480 hours, Table 1). Also, tillers which had not emerged prior to or during the vernalisation period became reproductive. These tillers must have been vernalised while still in the leaf sheath or as a very young bud at the base of the tiller, as the site of vernalisation is the growing point (Langer, 1972). If they had not required vernalisation, as previously discussed by Hare (1993a), some tillers from the unvernalised plants should have also produced seed heads.

In the field study, the majority of seed heads present at anthesis were produced by tillers that had emerged the previous late–summer and autumn, as found in field studies of other perennial temperate grass species (Langer and Lambert, 1959; Ryle, 1964; Hill and Watkin, 1975). These tall fescue tillers had received more than 60 days (1440 hours) of vernalisation (Table 6), the minimum period the controlled vernalisation study showed gave the most productive response (Table 1). However, at anthesis, 60% of tillers formed before the end of April remained vegetative (Table 4), in spite of having received exactly the same length of vernalisation as those tillers that became reproductive. In the controlled vernalisation study all tillers that received the full 60 days vernalisation became reproductive.

This anomaly may be explained in two ways. Firstly, Ryle (1961) stated that if cocksfoot tillers were shaded to less than 50% full sunlight they may fail to initiate. In the field study only a proportion of tillers may have received full sunlight throughout the winter and then initiated. Secondly, as suggested by Hare (1993b) there may be a maximum number of reproductive tillers per unit area that tall fescue plants can produce and support. In the field study the average number of reproductive tillers m^{-2} was 500 and it is possible that nutrient resources were not sufficient to support a greater number.

Tiller death was greatest in the spring period when stem elongation and canopy closure occurred. This was even after the autumn formed tillers had been treated with atrazine in the autumn, which does remove young tall fescue seedlings (Hare, 1993b) but not new tillers from older plants. Tiller death in spring could either be caused by a lack of light or a lack of carbohydrates and other nutrients such as nitrogen (Robson, 1968).

Both the controlled vernalisation and the field studies show that tall fescue plants do need a long period of winter cold (at least 1440 hours of temperatures under 8°C) in order to produce a sufficient number (approximately 500 m^{-2}) of reproductive tillers for seed production. Post–harvest management and autumn – winter defoliation of second year and older seed crops (Hare 1994) must therefore encourage and enhance the production of vegetative tillers in late summer and autumn for the maximum production of reproductive tillers in spring. Early sowing of new tall fescue seed crops in autumn (Hare, 1994) is advised so that new tillers can receive adequate vernalisation.

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REFERENCES

1. Bean, E.W. 1970. Short–day and low–temperature control of floral induction in *Festuca. Annals of Botany* 34: 57–66.
2. Hare, M.D. 1993a. Vernalisation and juvenility in tall fescue. *Journal of Applied Seed Production* 11: 73–79.
3. Hare, M.D. 1993b. Post–harvest and autumn management of tall fescue seed fields. *New Zealand Journal of Agricultural Research* 36: 407–408.
4. Hare, M.D. 1994. Autumn establishment of three New Zealand cultivars of tall fescue (*Festuca arundinacea* Schreb.) for seed production. *New Zealand Journal of Agricultural Research* 37: 11–17.
5. Hicks, D.H. and Mitchell, K.J. 1968. I. Interactions of day length and temperature on inflorescence emergence for *Festuca arundinacea* Schreb. variety Manade. *New Zealand Journal of Botany* 6: 86–93.
6. Hill, M.J. and Watkin, B.R. 1975. Seed production studies on perennial ryegrass, timothy and prairie grass. I. Effect of tiller age on tiller survival, ear emergence and seedhead components. *Journal of the British Grassland Society* 30: 63–71.
7. Langer, R.H.M. 1972. How grasses grow. The Institute of Biology's Studies in Biology, No. 34. Arnold, London. 60pp.
8. Langer, R.H.M. and Lambert, D.A. 1959. Ear–bearing capacity of tillers arising at different times in herbage grasses grown for seed. *Journal of the British Grassland Society* 14: 137–140.
9. Robson, M.J. 1968. The changing tiller population of spaced plants of S170 tall fescue (*Festuca arundinacea*). *Journal of Applied Ecology* 5: 575–590.
10. Ryle, G.J.A. 1964. The influence of date of origin of the shoot and level of nitrogen on ear size in three perennial grasses. *Annals of Applied Biology* 53: 311–323.
11. Tottman, D.R. and Broad, H. 1987. The decimal code for the growth stages of cereals, with illustrations. *Annals of Applied Biology* 110: 441–454.
12. Zadoks, J.C., Chang, T.T. and Konzak, C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research* 14: 415–421.