

[10.1071/CP23266](https://doi.org/10.1071/CP23266)

Crop & Pasture Science

Supplementary Material

Effects of heading date and *Epichloë* endophyte on persistence of diploid perennial ryegrass (*Lolium perenne*). 1. Heading date

Katherine N. Tozer^{A,}, David E. Hume^B, Catherine Cameron^A, Rose Greenfield^A, Tracy Dale^A, Wade J. Mace^B, Tony Craven^A, and Marty J. Faville^B*

^AAgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton 3240, New Zealand.

^BAgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North 4442, New Zealand.

*Correspondence to: Katherine N. Tozer AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton 3240, New Zealand Email: katherine.tozer@agresearch.co.nz

H x E paper 1 Supplementary material

Table S1. Percentage of the total number of tillers that were in the R0, R1, R2 and R4 reproductive development stages according to Moore *et al.* (1991). R0: boot stage; R1: first spikelet visible; R2: spikelets fully emerged; R3: inflorescence emerged; R4: anther emergence; R5: post-anthesis. None of the tillers were at the R3 or R5 stage. Assessments were done at the beginning and end of the long spring rotation for mid-heading cultivars (late Sept/early October to mid-November) and late-heading cultivars (late October – mid-December). Data were collected for late-heading cultivars only in December 2020. ‘-’ data were either insufficient for statistical analyses (October 2018 and 2019, and September 2020 and 2021) or not collected (December 2020). s.e.d.: standard error of difference.

Year	Development stage	Late Sept/early October				Late October				Mid-November				Mid-December			
		Mid	Late	s.e.d.	P value	Mid	Late	s.e.d.	P value	Mid	Late	s.e.d.	P value	Mid	Late	s.e.d.	P value
	Tillers (% of total)			
		3-Oct				31-Oct				20-Nov				7-Dec			
2018	R0	0	0	-	-	0	0	-	-	5.4	1.6	1.28	0.002	0.1	4.3	0.97	<0.001

2019

R1	0	0	-	-	0	0	-	-	14.2	0.1	1.48	<0.001	0.7	15.5	3.19	<0.001
R2	0	0	-	-	0	0	-	-	14.9	0.3	1.40	<0.001	0.0	9.9	3.48	<0.001
R4	0	0	-	-	0	0	-	-	0	0	-	-	0.0	4.8	1.49	<0.001
			3-Oct			30-Oct				19-Nov				16-Dec		
R0	0	0	-	-	2.8	0.5	0.61	<0.001	5.4	0.5	0.77	<0.001	1.3	9.9	1.86	<0.001
R1	0	0	-	-	1.5	0.0	0.68	0.021	23.6	0.3	1.96	<0.001	2.2	13.2	1.62	<0.001
R2	0	0	-	-	0.0	0.0	-	-	16.7	0.1	2.04	<0.001	0.6	14.6	2.06	<0.001
R4	0	0	-	-	0.0	0.0	-	-	7.1	0.0	1.28	<0.001	2.0	7.8	1.2	<0.001

		23-Sept				22-Oct				10-Nov				7-Dec			
2020	R0	0	0	-	-	3.2	0.1	0.98	0.002	18.2	10.7	3.25	0.012	-	10.2	-	-
	R1	0	0	-	-	0	0	-	-	18.8	0.3	1.99	<0.001	-	28.5	-	-
	R2	0	0	-	-	0	0	-	-	1.38	0.0	1.38	<0.001	-	16.1	-	-
	R4	0	0	-	-	0	0	-	-	0.0	0.0	-	-	-	8.1	-	-
		29-Sep				26-Oct				15-Nov				13-Dec			
2021	R0	0	0	-	-	0	0	-	-	9.3	2.5	1.88	<0.001	0.1	3.3	0.79	<0.001
	R1	0	0	-	-	0	0	-	-	20.7	1.1	2.62	<0.001	0.1	9.2	1.28	<0.001
	R2	0	0	-	-	0	0	-	-	0.5	0.0	0.48	<0.001	0.2	7.3	1.22	<0.001
	R4	0	0	-	-	0	0	-	-	0.0	0.0	-	-	0.0	2.4	-	-

Table S2. Effect of cultivar on percentage of vegetative, elongating and reproductive tillers, endophyte infection frequency, leaf stage, perennial ryegrass ground cover, tiller densities, herbage production, botanical composition and nutritive value. s.e.d., pooled standard error of difference. Bold text denotes when means are different at the level of $P < 0.05$ for comparisons between Bronsyn and Samson (mid-heading cultivars), or One50 and Rohan (late-heading cultivars). '-' data not collected and therefore statistical analysis could not be undertaken. Means for each cultivar are averaged over endophyte strain treatments (SE, AR37 and nea2/6) described in Table 2.

Measurement	Date	Bronsyn vs Samson			One50 vs Rohan			All cultivars	
		Bronsyn	Samson	P value	One50	Rohan	P value	s.e.d.	P value
Percentage of vegetative tillers (%)	3 October 2018	82	92	0.022	88	88	0.830	4.0	0.123
	22 October 2020	66	75	0.025	89	87	0.700	3.6	<0.001
	7 December 2020	-	-	-	43	33	0.005	2.8	-
Percentage of elongating tillers (%)	3 October 2018	18	8	0.022	12	12	0.830	4.0	0.123
	31 October 2018	24	34	0.038	27	21	0.181	4.8	0.028
	16 December 2019	1	0	0.537	4	1	0.002	1.0	0.004
Percentage of reproductive tillers (%)	30 October 2019	6	3	0.006	0	1	0.900	1.1	<0.001

	16 December 2019	4	8	0.235	39	48	0.026	3.9	<0.001
	22 October 2020	5	1	<0.001	0	0	0.798	1.1	<0.001
	7 December 2020	-	-	-	56	65	0.027	4.3	-
Endophyte infection frequency (%)	28 May 2018	95	90	0.022	91	92	0.593	2.1	0.097
	3 October 2018	98	93	0.010	88	93	0.008	1.8	<0.001
	13 May 2019	97	94	0.152	91	96	0.022	2.1	0.057
	6 May 2021	98	95	0.015	92	94	0.120	1.3	<0.001
Leaf stage (number of new leaves)	3 October 2018	2.8	2.8	0.587	2.7	2.9	0.002	0.07	0.025
	17 December 2018	2.6	2.7	0.152	2.6	2.9	0.001	0.08	0.002
	13 May 2019	2.6	2.5	0.285	2.5	2.7	0.039	0.07	0.108
	27 June 2019	2.7	2.6	0.571	2.6	2.7	0.047	0.05	0.187
	13 December 2021	2.6	2.5	0.586	2.6	2.7	<0.001	0.05	0.002
Perennial ryegrass ground cover (out of 10)	17 December 2018	7.6	7.2	0.234	7.7	7.1	0.040	0.30	0.119
	21 January 2019	6.9	5.7	0.002	7.6	7.4	0.730	0.35	<0.001

5 August 2019	8.2	7.9	0.055	8.7	8.3	0.005	0.14	<0.001
6 September 2019	8.2	7.8	0.043	8.3	8.3	1.00	0.16	0.006
16 December 2019	7.5	7.2	0.019	7.9	8.0	0.400	0.15	<0.001
27 April 2020	7.4	6.4	0.006	7.8	7.8	0.901	0.33	<0.001
8 June 2020	7.4	6.4	0.009	7.9	7.9	0.987	0.35	<0.001
5 August 2020	7.2	6.7	0.098	7.9	7.2	0.013	0.27	0.002
31 August 2020	7.3	6.6	0.021	7.8	7.7	0.675	0.30	<0.001
23 September 2020	7.4	6.8	0.006	8.0	7.6	0.133	0.23	<0.001
13 January 2021	6.1	4.9	0.012	5.9	6.4	0.307	0.46	0.015
10 March 2021	3.7	2.7	0.048	2.7	2.3	0.380	0.47	0.034
14 April 2021	5.1	3.4	0.007	4.2	3.4	0.206	0.60	0.018
1 June 2021	6.8	6.0	0.015	6.8	6.8	1.00	0.31	0.040
2 August 2021	6.5	5.6	0.013	6.8	6.8	0.945	0.32	0.002
13 December 2021	6.7	5.3	<0.001	7.1	7.2	0.746	0.35	<0.001

	1 February 2022	4.4	3.1	0.002	4.6	4.7	0.771	0.38	<0.001
	28 February 2022	3.5	2.4	0.005	3.4	3.1	0.462	0.37	0.020
Tiller density (tillers m ⁻²)	3 October 2018	5940	5390	0.165	5660	6470	0.043	382	0.031
	4 March 2019	3260	3890	0.025	3230	4320	<0.001	264	<0.001
	3 October 2019	4860	5440	0.290	5740	6870	0.043	535	0.004
	24 February 2020	2910	1770	0.036	3290	4170	0.105	522	<0.001
	23 September 2020	6370	5750	0.281	6730	8110	0.021	569	0.001
	13 January 2021	4390	3070	0.017	2450	2920	0.375	526	0.007
	14 April 2021	3880	3430	0.121	3680	4420	0.014	286	0.014
	25 May 2022	2650	1530	0.005	1970	3300	0.001	371	<0.001
Seasonal herbage production (kg DM ha ⁻¹)	Winter 2021	1160	1060	0.019	1150	1190	0.279	38	0.015
	Winter (4-year mean)	2050	1610	0.008	2130	1940	0.249	158	0.012
	Spring (4-year mean)	4730	4270	0.013	3900	3910	0.964	176	<0.001
Perennial ryegrass content (% in total DM)	17 December 2018	82	77	0.035	84	83	0.716	2.6	0.043

	16 December 2019	38	26	0.010	52	49	0.449	4.4	<0.001
	14 April 2021	48	35	0.015	38	42	0.406	5.2	0.062
	29 September 2021	56	46	0.026	59	60	0.806	4.2	0.008
	13 December 2021	58	35	<0.001	49	46	0.592	5.1	<0.001
Other grass content (% in total DM)	17 December 2018	1	1	0.524	3	1	0.023	0.9	0.079
	24 February 2020	9	7	0.280	6	12	0.030	2.6	0.129
	28 February 2022	40	27	0.017	18	18	0.945	5.2	0.020
Legume content (% in total DM)	13 January 2021	2	4	0.003	1	2	0.220	0.7	<0.001
Broadleaf content (% in total DM)	16 December 2019	49	64	0.008	33	39	0.264	5.1	<0.001
	13 December 2021	22	40	<0.001	25	26	0.856	4.4	<0.001
	28 February 2022	28	42	0.017	38	39	0.871	5.5	0.060
	25 May 2022	22	34	0.012	25	26	0.874	4.3	0.058
Dead vegetation content (% in total DM)	16 December 2019	10	8	0.128	10	6	0.031	1.4	0.035
Metabolisable energy (MJ kg DM ⁻¹)	3 October 2018	12.1	12.2	0.458	12.1	12.4	0.005	0.11	0.013

	4 March 2019	8.6	9.0	0.007	9.1	9.2	0.449	0.16	<0.001
	1 June 2021	12.4	12.8	0.040	12.7	12.6	0.368	0.16	0.141
	28 February 2022	10.8	11.4	0.006	11.0	10.7	0.128	0.20	0.008
Crude protein (% of total DM)	3 October 2018	15.6	14.9	0.171	14.7	17.2	<0.001	0.49	<0.001
	8 June 2020	20.4	21.7	0.022	20.2	19.3	0.087	0.53	<0.001
	5 August 2020	22.8	24.5	0.035	23.5	23.8	0.730	0.75	0.203
	14 April 2021	23.5	24.1	0.094	22.6	23.4	0.033	0.35	0.004
	2 August 2021	23.0	24.2	0.027	24.9	24.0	0.106	0.50	0.010
	28 February 2022	18.7	19.9	0.008	19.1	19.1	1.00	0.43	0.048
	25 May 2022	17.6	18.0	0.584	16.9	18.6	0.013	0.63	0.105
Neutral detergent fibre (% of total DM)	3 October 2018	45.5	45.3	0.652	46.2	44.0	<0.001	0.55	0.003
	17 December 2018	50.1	50.1	0.984	52.5	51.0	0.006	0.49	<0.001
	13 May 2019	41.8	42.0	0.744	42.3	41.0	0.037	0.58	0.166
	4 October 2019	43.7	43.8	0.830	45.1	43.8	0.008	0.46	0.029

	24 February 2020	53.8	55.0	0.151	54.7	53.0	0.042	0.81	0.064
	8 June 2020	41.2	41.3	0.907	44.0	41.8	0.016	0.85	0.018
	5 August 2020	39.9	39.1	0.233	42.0	40.5	0.020	0.59	<0.001
	1 June 2021	36.6	34.1	0.002	35.5	35.5	0.958	0.76	0.015
	2 August 2021	36.4	34.4	0.001	35.8	35.7	0.985	0.52	0.004
	13 December 2021	46.4	43.3	0.029	50.5	50.2	0.789	1.33	<0.001
	28 February 2022	42.9	38.8	0.003	40.1	42.8	0.039	1.25	0.014
Ash (% of total DM)	3 October 2018	8.8	8.3	0.013	8.8	8.9	0.594	0.20	0.023
	13 December 2018	10.8	10.2	0.015	8.5	8.8	0.189	0.22	<0.001
	4 March 2019	11.7	10.6	0.004	10.8	10.6	0.496	0.33	0.004
	5 August 2019	10.3	11.3	0.017	11.3	11.5	0.715	0.43	0.019
