

**Supplementary Material**

**Effects of heading date and *Epichloë* endophyte on persistence of diploid perennial ryegrass (*Lolium perenne*). 2. Endophyte strain and interactions with heading date**

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## Supplementary material

**Table S1.** Interactions at  $P < 0.05$  between heading date and endophyte for metabolisable energy and alkaloid concentrations of lolitrem B and peramine. Data are the mean of two heading date treatments. For each measurement, means are in bold when there are differences at the level of  $P \leq 0.05$  between mid- and late-heading cultivars for each endophyte strain. s.e.d.: standard error of difference. '-' not applicable.

Measurement	Month/season	AR37		nea2/6		SE		s.e.d.	P value
		Mid	Late	Mid	Late	Mid	Late		
Metabolisable energy (MJ kg DM <sup>-1</sup> )	5 August 2019	<b>12.8</b>	<b>12.3</b>	12.6	12.7	12.6	12.4	0.17	0.024
	28 February 2022	<b>11.4</b>	<b>10.5</b>	11.0	10.9	10.8	11.1	0.33	0.046
Lolitrems B concentration (mg kg <sup>-1</sup> )	17 December 2018	-	-	0.13	0.30	<b>1.79</b>	<b>2.72</b>	0.233	0.029
	4 March 2019	-	-	0.33	0.46	<b>2.15</b>	<b>1.56</b>	0.220	0.038
	23 September 2020	-	-	0.00	0.00	<b>0.25</b>	<b>0.30</b>	0.014	0.016
	13 January 2021	-	-	0.21	0.30	<b>2.43</b>	<b>1.75</b>	0.190	0.010
	13 December 2021	-	-	0.14	0.36	<b>1.87</b>	<b>3.60</b>	0.199	<0.001

Peramine concentration (mg kg <sup>-1</sup> )	4 March 2019	-	-	3.7	3.2	<b>11.8</b>	<b>6.8</b>	0.61	<0.001
	2 October 2019	-	-	4.9	5.6	<b>12.5</b>	<b>10.3</b>	1.06	0.050
	16 December 2019	-	-	4.0	5.4	<b>16.5</b>	<b>13.2</b>	1.52	0.030
	24 February 2020	-	-	4.2	4.1	<b>13.9</b>	<b>10.6</b>	0.88	0.010
	12 January 2021	-	-	6.4	5.4	<b>21.9</b>	<b>12.9</b>	1.29	<0.001
	1 June 2021	-	-	5.9	7.3	<b>21.4</b>	<b>17.4</b>	1.75	0.021
	2 August 2021	-	-	<b>4.3</b>	<b>6.0</b>	13.2	12.2	0.79	0.023
	27 September 2021	-	-	<b>4.4</b>	<b>6.1</b>	<b>14.2</b>	<b>13.0</b>	0.53	0.011
	28 February 2022	-	-	3.4	2.9	<b>12.4</b>	<b>8.8</b>	1.10	0.047

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**Table S2.** Effect of cultivar on alkaloid concentrations of total epoxyjanthitrems, lolitrem B, ergovaline and peramine. 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 1.

Measurement	Date	Bronsyn vs Samson			One50 vs Rohan			All cultivars	
		Bronsyn	Samson	P value	One50	Rohan	P value	s.e.d.	P value
Total epoxyjanthitrems (mg kg <sup>-1</sup> )	3 October 2018	7.6	5.5	0.255	<b>10.2</b>	<b>5.1</b>	0.012	1.70	0.054
	5 August 2019	1.5	1.5	0.704	<b>2.0</b>	<b>1.3</b>	0.003	0.19	0.027
	3 October 2019	4.3	4.9	0.193	<b>6.3</b>	<b>4.0</b>	0.001	0.47	0.004
	16 December 2019	<b>21.0</b>	<b>29.6</b>	0.037	27.7	31.1	0.375	3.74	0.058
	8 June 2020	8.5	7.3	0.288	<b>11.9</b>	<b>7.8</b>	0.002	1.03	0.006
	5 August 2020	1.8	1.5	0.444	<b>3.4</b>	<b>1.6</b>	0.001	0.42	0.004
	23 September 2020	2.1	2.1	0.958	<b>3.7</b>	<b>2.1</b>	0.013	0.55	0.041
	13 January 2021	<b>24.0</b>	<b>31.9</b>	0.013	15.1	15.4	0.922	2.60	<0.001
	2 August 2021	<b>0.94</b>	<b>0.67</b>	0.049	<b>1.48</b>	<b>1.20</b>	0.043	0.121	<0.001
	25 May 2022	35.9	30.7	0.074	<b>37.3</b>	<b>27.9</b>	0.005	2.66	0.020

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Lolitrem B (mg kg <sup>-1</sup> )	3 October 2018	0.73	0.76	0.859	<b>1.07</b>	<b>0.62</b>	0.022	0.183	0.859
	17 December 2018	1.16	0.79	0.082	<b>2.48</b>	<b>1.61</b>	<0.001	0.201	<0.001
	4 March 2019	1.20	1.24	0.846	<b>1.78</b>	<b>0.92</b>	0.002	0.239	0.034
	13 May 2019	0.74	0.55	0.150	<b>1.10</b>	<b>0.73</b>	0.008	0.128	0.007
	3 October 2019	0.39	0.31	0.212	<b>0.72</b>	<b>0.42</b>	<0.001	0.065	<0.001
	16 December 2019	1.41	1.69	0.081	<b>3.40</b>	<b>2.34</b>	<0.001	0.155	<0.001
	24 February 2020	1.59	1.31	0.087	<b>2.40</b>	<b>1.46</b>	<0.001	0.159	<0.001
	8 June 2020	0.54	0.41	0.165	<b>0.83</b>	<b>0.56</b>	0.008	0.092	0.006
	5 August 2020	0.09	0.08	0.777	<b>0.20</b>	<b>0.10</b>	<0.001	0.022	<0.001
	23 September 2020	0.12	0.13	0.460	<b>0.29</b>	<b>0.15</b>	<0.001	0.013	<0.001
	13 January 2021	1.36	1.28	0.698	<b>1.84</b>	<b>0.98</b>	<0.001	0.203	0.006
	14 April 2021	1.10	1.03	0.766	<b>1.66</b>	<b>0.86</b>	0.002	0.222	0.031
	1 June 2021	0.25	0.22	0.428	<b>0.44</b>	<b>0.25</b>	<0.001	0.035	<0.001
	2 August 2021	0.09	0.07	0.155	<b>0.16</b>	<b>0.09</b>	<0.001	0.014	<0.001

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	29 September 2021	0.16	0.19	0.375	<b>0.47</b>	<b>0.24</b>	<0.001	0.030	<0.001
	13 December 2021	1.05	0.96	0.616	<b>3.34</b>	<b>2.12</b>	<0.001	0.190	<0.001
	28 February 2022	0.56	0.40	0.084	<b>0.72</b>	<b>0.54</b>	0.044	0.087	0.021
	25 May 2022	2.27	2.23	0.886	<b>3.88</b>	<b>2.43</b>	<0.001	0.338	0.001
Ergovaline (mg kg <sup>-1</sup> )	3 October 2018	<b>0.57</b>	<b>0.31</b>	0.027	0.19	0.31	0.287	0.110	0.015
	17 December 2018	0.20	0.14	0.303	<b>0.21</b>	<b>0.40</b>	0.003	0.056	<0.001
	24 February 2020	<b>1.38</b>	<b>0.82</b>	0.004	0.46	0.65	0.272	0.173	<0.001
	13 January 2021	<b>0.30</b>	<b>0.18</b>	<0.001	0.05	0.08	0.203	0.026	<0.001
	14 April 2021	0.27	0.27	0.965	<b>0.14</b>	<b>0.24</b>	0.001	0.027	0.001
	1 June 2021	<b>0.43</b>	<b>0.32</b>	0.005	0.22	0.27	0.126	0.037	<0.001
	13 December 2021	0.51	0.38	0.162	<b>0.27</b>	<b>0.45</b>	0.044	0.088	0.100
	28 February 2022	0.35	0.29	0.078	<b>0.24</b>	<b>0.31</b>	0.025	0.030	0.028
	25 May 2022	<b>0.88</b>	<b>0.55</b>	<0.001	<b>0.29</b>	<b>0.62</b>	<0.001	0.076	<0.001
Peramine (mg kg <sup>-1</sup> )	17 December 2018	7.7	7.4	0.706	<b>12.0</b>	<b>9.7</b>	0.004	0.73	<0.001

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13 May 2019	<b>13.9</b>	<b>10.5</b>	0.005	13.0	12.8	0.884	1.09	0.018
24 February 2020	<b>10.1</b>	<b>8.0</b>	0.001	<b>9.7</b>	<b>7.8</b>	0.002	0.56	<0.001
5 August 2020	<b>9.2</b>	<b>8.0</b>	0.042	11.0	10.0	0.087	0.55	<0.001
13 January 2021	<b>15.4</b>	<b>13.2</b>	0.032	10.8	10.1	0.464	0.97	<0.001
14 April 2021	<b>17.2</b>	<b>14.8</b>	0.019	17.1	15.7	0.136	0.94	0.040
1 June 2021	<b>15.2</b>	<b>12.0</b>	0.006	15.4	13.4	0.067	1.05	0.011
29 September 2021	9.3	9.3	0.918	<b>12.3</b>	<b>9.9</b>	0.006	0.76	0.009
13 December 2021	13.1	11.2	0.106	<b>22.0</b>	<b>17.8</b>	0.001	1.14	<0.001
28 February 2022	<b>8.7</b>	<b>7.1</b>	0.043	7.30	6.7	0.398	0.74	0.039
25 May 2022	17.1	15.2	0.088	<b>20.2</b>	<b>17.2</b>	0.009	1.06	0.003

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**Table S3.** Abundance of clover root weevil larvae, black beetle adults, white fringe weevil larvae, wireworm adults and earthworms, randomly sampled from internal buffers (mean  $\pm$ sem (standard error of the mean)).

Date	Abundance (m <sup>-2</sup> )				
	Clover root weevil larvae	Black beetle adult	White fringe weevil larvae	Wireworm adult	Earthworm <i>spp.</i>
19 March 2019	0	0	15 ( $\pm$ 7)	8 ( $\pm$ 4)	168 ( $\pm$ 26)
28 April 2021	48 ( $\pm$ 27)	10 ( $\pm$ 6)	20 ( $\pm$ 10)	8 ( $\pm$ 4)	720 ( $\pm$ 81)