Elevated atmospheric CO_2 alters heading date of perennial ryegrass

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Abstract
Carbon dioxide (CO_2) levels are increasing globally and affect plant growth and development. Time to flowering, commonly referred to as heading date, has been identified as a key indicator of the quality and nutritional value of ryegrass. Recent research on annual grasses indicates that elevated CO_2 levels can delay heading date, however significant data for perennial ryegrass is lacking. We exposed currently available ryegrass cultivars to the CO_2 concentration expected in 2050 (500 ppm) and found significant changes in heading date with delays and advances of up to 10 days. Over all the cultivars the breadth of heading date was more than doubled, offering potentially new possibilities for cultivar choice for specific environments and systems.

Keywords*:* *Lolium perenne*, climate change, plant phenology, phosphorus, nitrogen

Introduction
An increasing concentration of CO_2 in the atmosphere, the most predictable aspect of global climate change and the concentration in the New Zealand atmosphere, has increased by almost 20% over the last 40 years. Because a higher concentration of CO_2 results in greater photosynthesis, the expectation is that elevated CO_2 will stimulate pasture production (Lüscher *et al.* 2005) in New Zealand (Lieffering *et al.* 2012). Perennial ryegrass (*Lolium perenne* L.) is the grass species preferred by New Zealand's pastoral industries mainly because of its rapid establishment, good yields and high digestibility (Easton *et al.* 2011). It is highly likely that ryegrass will continue to be the dominant pasture feed base for use in New Zealand's dairy and drystock farming systems in the future when any effects of elevated CO_2 will be more strongly expressed. Heading date is an important agronomic trait for farmers to consider during ryegrass selection. The change from vegetative to reproductive growth signifies a change in biomass partitioning, an increase in lignin content and subsequent drop in nutritive value (Skøt *et al.* 2005). Early heading cultivars provide good growth in the early part of the season while late heading cultivars provide higher quality feed through late spring and summer. Daylength and temperature have been identified as the major triggers for reproductive growth, however recent research on annual ryegrass demonstrated that elevated CO_2 delayed flowering in annual ryegrass (Cleland *et al.* 2006). Here we examine whether elevated CO_2 has a similar effect on perennial ryegrass using a diverse range of ryegrass populations grown in a field laboratory where the plants were exposed to the atmospheric CO_2 concentration expected in 2050.

Methods
The experiment was conducted at the Free-air CO_2 Enrichment (FACE) site located at Flock House, Bulls, Rangitikei. The FACE allows areas of pasture to be exposed to higher CO_2 concentrations without the necessity to have enclosures like greenhouses or open-top chambers. The site consists of six experimental 12 m diameter areas that receive either ambient CO_2 (398 ppm) or the CO_2 concentration expected in 2050 (500 ppm). A further description can be found in Newton *et al.* (2006).

The experiment tested the effects of ryegrass cultivar and genotype, CO_2 elevation, the presence of endophyte and the effect of phosphorus (P) fertiliser on heading date.

We used four genotypes each of seven perennial ryegrass cultivars and one advanced breeding line (Table 1); for convenience these are hereafter all described as cultivars. The cultivars were selected for their strong performance under soil moisture limitation for use in a broader study of elevated CO_2 effects and so were not selected specifically to cover a range of heading dates.

Plants were grown from endophyte-infected seed at AgResearch Grasslands, Palmerston North. When plants had grown approximately 10 tillers they were split into two clonal replicates. One clone was used to generate endophyte-free plants using methods described by Latch & Christensen (1982). Immunodetection (Simpson *et al.* 2012) and microscopy (Latch & Christensen 1982) confirmed the endophyte status of each plant after the fungicide treatment. Plants were cloned further to generate a total of 24 copies per genotype and allocated to treatments (Table 2).

To establish the experiment, small plants of approximately twenty tillers, with laminae trimmed to 20 mm above the ligule were transplanted into bare soil, within each experimental ring, at the FACE site on 15 July 2013 in a completely randomised row-column grid design (350 mm spacing), optimised to avoid clonal

replicates from being present twice in the same row or column as described by Hatier *et al.* (2014). The plants were surrounded by PVC pipes (200 mm long and 110 mm diameter) to restrict nutrients to individual plants. After establishment the plants were cut to 20 mm above ground level on 30 September 2013. A weed mat was installed between the plants to minimise weed establishment. Rings were irrigated in pairs at a rate of 10 mm per ring each morning. Irrigation was supplied via a computer controlled sprinkler, installed in the middle of each ring.

At planting, phosphorus was applied in solution as monopotassic phosphate (KH_2PO_4) at 0 or 35 kg P ha^{-1} to half the plants and all plants received nitrogen fertiliser as urea ($CO(NH_2)_2$) in solution at a rate of 50 kg N ha^{-1}. Thereafter the fertiliser treatments were applied every 56 days starting on 3 September 2013.

As it was not possible to destructively harvest the plants during the heading date recording period we assessed dry matter (DM) and visual signs of nutrient deficiency using a visual scoring system (1 to 5 scale) every 28 days; tiller numbers were also counted at this time. A final DM cut was taken on 18 December 2013, when the recording period had finished. All foliage samples were dried at 60°C for 48 hours and weighed.

Heading date was monitored from the time of appearance of the first reproductive tillers until the majority of the plants had reached full maturity. Plants were checked every second day for seed head emergence. The date of emergence of three heads per plant was recorded as the heading date.

The effect of treatments on heading date was tested using a general ANOVA test in Genstat 14 for Windows.

Table 1 Commercial name, endophyte strain and typical heading behaviour of perennial ryegrass plants used in this experiment

Cultivar	Endophyte	Heading behaviour
Alto	AR37	Late
Avalon	AR1	Late
Banquet II ^	Endo 5	Late
Bealey^	NEA2	Late
Commando	AR37	Early
GA194#	AR37	Mid
One50	AR37	Late
Trojan	NEA2	Late

^ Tetraploid
# Breeding line

Table 2 Plant treatment levels and running total of plant numbers required for the experiment

Treatments	Levels	Plants required
Cultivars	8	8
Genotypes	4	32
CO_2 concentrations	6	192
Endophyte status	2	384
Phosphorus concentrations	2	768

Table 3 Summary of ANOVA analysis of heading date for perennial ryegrass populations exposed to elevated CO2 at the FACE site. CO_2 = CO_2 treatment, C = cultivar, E = endophyte infection, P = phosphorus

	Df	Sum Sq	Mean Sq	F Value	P value
CO_2	1	298.1	298.11	3.1702	0.108
C	7	17915.5	2559.36	27.2169	**<0.001**
E	1	0.1	0.11	0.0012	0.818
P	1	5.0	4.97	0.0529	0.852
CO_2 × C	7	4964.6	709.23	7.5422	**<0.001**
CO_2 × E	1	0.5	0.47	0.0050	0.958
C × E	7	458.6	65.52	0.6968	0.304
CO_2 × P	1	74.0	73.98	0.7867	0.425
C × P	7	743.9	106.28	1.1302	0.069
E × P	1	156.2	156.23	1.6614	0.169
CO_2 × C × E	7	436.1	62.30	0.6625	0.625
CO_2 × C × P	7	439.0	62.71	0.6669	0.664
CO_2 × E × P	1	87.4	87.45	0.9299	0.266
C × E × P	7	207.3	29.61	0.3149	0.457
CO_2 × C × E × P	7	293.8	41.97	0.4463	0.741

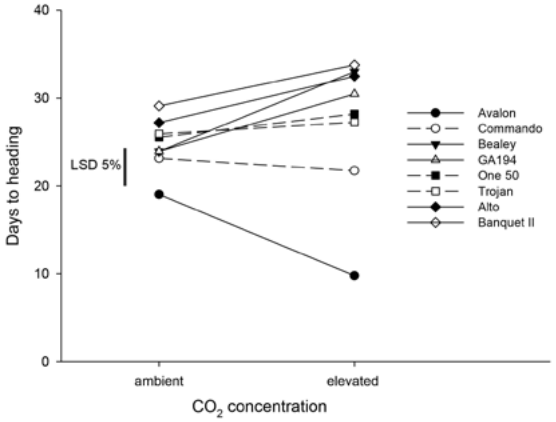

Figure 1 Reaction norms for heading date (days to emergence (0 = 29 October 2013) of three ears) for perennial ryegrass cultivars under ambient (398 ppm) and elevated (500 ppm) CO_2 concentrations. Solid lines indicates significant differences ($P<0.05$) between CO_2 treatments; dashed lines indicates differences were non-significant. The vertical bar is the LSD.

Means were separated by Fishers Protected LSD (5%) test. Dry matter production, live tiller number and nutrient stress scores were tested using a Linear Mixed model analysis with multiple permutation tests to validate results.

Results

There was a significant cultivar × CO_2 interaction for the time of heading ($P\leq0.001$) but no effect of endophyte status, P or any further interactions (Table 3). There was significantly earlier heading of the cultivar 'Avalon' under elevated CO_2, but significantly delayed heading of the cultivars 'GA194', 'Alto', 'Banquet II' and 'Bealey' (Figure 1). The difference in heading dates within a cultivar was up to 10 days.

The cultivars that headed earlier under ambient CO_2 tended to head even earlier under elevated CO_2, but the later heading cultivars at ambient delayed heading further under elevated CO_2 (Figure 1). This increased the span of heading dates across cultivars from about 10 days to 26 days. With the exception of 'Avalon' the cultivar heading dates under ambient CO_2 followed their expected behaviour (Table 1) with the early and mid cultivars being earlier than the late cultivars, although the differences were marginal in some cases (Figure 1).

There was no measured or observed difference in DM production, live tiller number or evidence of nutrient deficiency in response to CO_2 elevation during the experimental period (data not shown).

Discussion

The change from vegetative to reproductive growth in perennial ryegrass causes a change in resource utilisation, allocation and photosynthesis, and biomass production increases when plants become reproductive (Skøt *et al.* 2005). However, this reproductive growth is associated with an increase in lignified tissue and consequent drop in nutritive value (metabolisable energy) (Kemp & Culvenor 1994). Consequently, cultivars with different heading dates have different agronomic value. Earlier heading can result in production benefits from earlier spring growth (Burke *et al.* 2007; Kennedy & O'Donovan 2014) and are valuable in environments where summer growth is unreliable due to variable soil moisture content (Kemp & Culvenor 1994). By contrast, late heading cultivars are used to maintain quality longer into the season and have become popular in areas where water is non-limiting and high quality forage is desired in late spring/early summer (Edwards & Bryant 2011; Stewart *et al.* 2014). In this study we found that under the CO_2 concentration expected in about 35 years' time the heading date of five of the eight ryegrass cultivars tested was significantly changed. The difference in heading dates for individual cultivars was about 10 days but the range covered under ambient increased from 10 days at ambient to 26 days under elevated CO_2. This broadening of heading times offers additional possibilities for cultivar choice to suit specific environments and systems. Further investigation is required to determine whether these changes might be modified by projected changes in temperature and rainfall and to identify consequences of these changes for patterns of forage production and quality.

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