Seasonal differences in the capacity of perennial ryegrass to respond to gibberellin explained

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Abstract

Perennial ryegrass plants (*Lolium perenne*) were taken from an established field at two different stages in the season (mid-winter and again at mid-summer). They were then grown in a controlled environment to both "lock in" their contrasting developmental states and to look at the role of nitrogen supply, temperature, and developmental state separately to evaluate the potential of plants to respond to exogenous application of gibberellin. Responses to exogenous gibberellin (gibberellic acid, GA) were significant but were far smaller in summer-derived than winter-derived plants. The major difference in response to GA (compared with controls) between winter-derived and summer-derived plants suggests that seasonal changes in plant developmental state have a major effect in the field on the capacity for the plants to respond to exogenous GA application. This effect is greater than that of temperature and N availability. This raises new prospects for making sustained increases in plant growth, but only if the fundamental mechanisms by which plants control their responses to environmental signals (e.g., temperature and soil N status) can be understood. The role of gibberellins (endogenous as well as externally applied) in changes in plant growth strategy presents a new challenge for forage plant science.

Introduction

Gibberellins have been proposed in New Zealand as a means to increase forage supply, and improve the match between forage supply and feed demand in pastoral systems (Beukes *et al*. 2012).

Gibberellins (gibberellic acid, GA) are a family of naturally occurring plant hormones which regulate plant growth and influence various developmental processes including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, and leaf and fruit senescence (reviewed in Matthew *et al.* 2009). These highly bioactive hormones have been commercially available and widely used in plant physiological studies from the 1950s to the 1970s (Leben & Barton 1957). The bulk synthesis of gibberellins has made application increasingly affordable.

Field-based research focusing on the use of GA on grassland species has shown in many cases (see Matthew *et al.* 2009) that GA application can increase overall forage dry matter (DM) production. There is, however, clear evidence that the stimulation is greatest early in the growing season, and that GA is less effective in mid-summer and early autumn (see Leben *et al.* 1959; Williams & Arnold 1964; Matthew *et al*. 2009).

Possible causes for different seasonal responses might be:

i. seasonal differences in the availability of nutrients (notably N) sufficient to support any extra growth. This has a bearing on fertiliser recommendations when using GA;
ii. seasonal differences in temperature. Low temperature will clearly limit growth, though industry recommendations suggest GA is most effective at times when growth is limited by low temperature (NuFarm 2011);
iii. major seasonal changes in the developmental state (e.g. cycle of vegetative growth/flowering) and associated growth strategy (Parsons *et al.* 2011) in our widely used perennial grasses; and
iv. the possible effects of repeat applications of GA.

To gather insights into each of these hypotheses we took plants from the field and grew them in a common controlled environment to "lock in" two contrasting developmental states. We then measured the roles of nitrogen supply, temperature, and developmental state, separately, on the potential of plants to respond to GA.

Materials and Methods

Plants and growing conditions

Perennial ryegrass plants (cv. 'Aberdart') were collected from an established dairy pasture at two different stages in the growing season: i) June 2011; these being "winter-derived" and, ii) December 2011; these being "summer-derived" plants. Plants had all original soil washed from their roots and were separated into healthy, consistently sized plants consisting of approximately

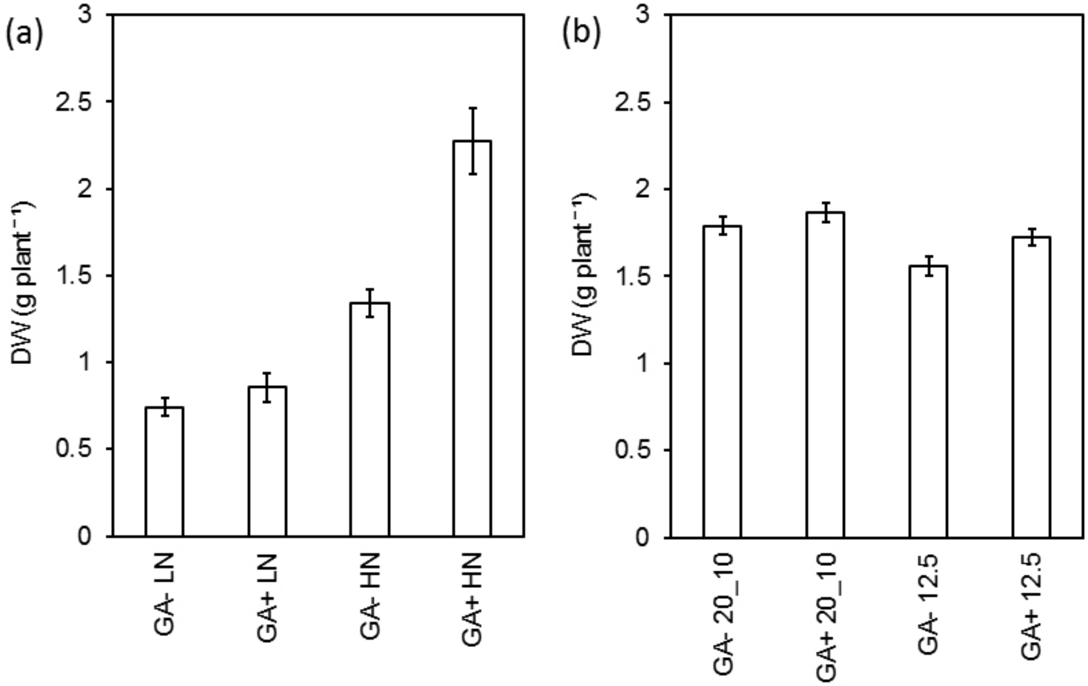

Figure 1. (a) Harvested yield (dry weight above 6 cm after 3 weeks' regrowth) of winter-derived ryegrass plants grown at high N (HN) and low N supply (LN) at 12.5°C and sprayed with or without gibberellic acid (GA+, GA-). (b) Harvested yield (dry weight above 6 cm after 3 weeks' regrowth) of summer-derived plants grown at high N supply at either 20/10°C or 12.5°C, and sprayed with or without gibberellic acid (GA+, GA-). Vertical bars are standard errors of the means.

10 tillers. These were transferred to plastic pots and planted in a 2:1 sand/clay medium with a known low nutrient retaining capacity. The plants were grown in a controlled environment (New Zealand Controlled Environment Laboratory, Plant and Food Research, Palmerston North). All plants were maintained on matting in drained plastic trays, which were flood sub-irrigated with tap water every second day to minimise any accumulation of nutrients within the pots.

The plants were transferred to growth rooms (for environmental conditions see below) and were clipped to 6 cm above ground every 4 weeks for winter-derived plants and every 3 weeks for summer-derived plants (anticipating faster growth at the higher day length/light energy receipt). The plants were allowed to establish during at least two full defoliation cycles before GA treatments were applied and measurements were taken.

Plants collected in winter (960 in total) were grown at 12.5°C and in short days (8 hours light) critical to sustain the plants in an induced but not initiated (Cooper 1952; Ryle & Langer 1963) winter developmental state. Radiation flux density was 620 µmols m^{-2} s^{-1}. Plants were supplied every second day with either "high" (50% of the plant pots) or "low" (50% of the plant pots) mineral N solution, using half-strength Hoagland solution; 60 ml per plant/pot at 9 m*M* N or adjusted to 2.25 m*M* N to sustain contrasting N status (Rasmussen *et al.* 2007).

Plants collected in summer (960 in total) were grown at two temperature regimes: 12.5°C throughout (as above), and 20°C/10°C for day/night. All plants were supplied with the "high" mineral N solution and were grown at long days (14 hours light), critical to sustain the plants in a "summer", vegetative state. Radiation flux density was again 620 µmols m^{-2} s^{-1}.

Exogenous gibberellic acid treatment

i) In the winter-derived plants, on one occasion, half of the high N plants and half of the low N plants (i.e. 480 plants) were sprayed with exogenous gibberellin (ProGibb-SG™), and surfactant (Contact™), 4 days after defoliation. Application rates were equal to the recommended 20 g/ha ProGibb-SG, 40% active ingredient, in a fine foliar spray of 100 L/ha containing 20 ml/100 L surfactant. The control received only surfactant.

ii) For the summer-derived plants, on one occasion, half of a subset of the 12.5°C/12.5°C plants and half of the 20°C/10°C plants (i.e. 160 plants) were sprayed with exogenous gibberellin and surfactant solution after defoliation, as above. Again, the control received only surfactant.

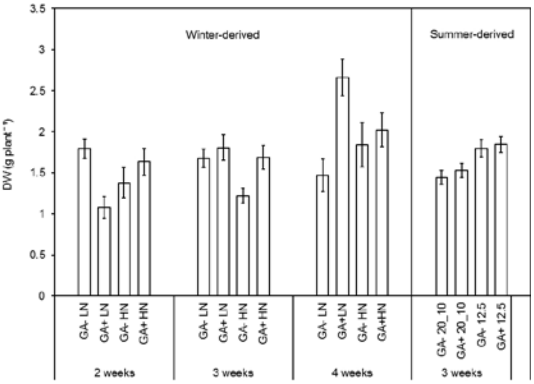

Figure 2. Dry weights of roots after 2, 3 and 4 weeks' regrowth of winter derived plants grown at high N (HN) and low N supply (LN) at 12.5°C and sprayed with or without gibberellic acid (GA+, GA-). Dry weights of roots after 3 weeks' regrowth of summer-derived plants grown at high N supply at either 20/10°C or 12.5°C, and sprayed with or without gibberellic acid (GA+, GA-). Vertical bars are standard errors of the means.

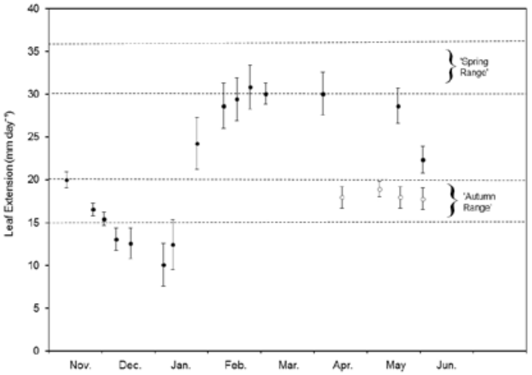

Figure 3. Changes in potential rate of leaf extension in plants taken from the field, and measured indoors at 15°C, throughout autumn 1976 to spring 1977 (solid symbols) and plants growing in spring which had not experienced a previous winter (open symbols) (after Parsons & Robson 1980). N.B. data from Northern Hemisphere. Vertical bars are standard errors of the means.

Sampling and results analysis

At intervals (2, 3, 4 weeks) during regrowth in winter-derived plants, and at 3 weeks for summer-derived plants, plants were dissected into herbage (cut 6 cm above the ground), stubble (remaining shoot below 6 cm), and roots (washed from soil medium).

We used a 2-way ANOVA to analyse results, using individual plants (each plant in a separate pot) as the unit of replication (see Newman *et al.* 1997) to describe the main effects, and interaction of GA and N (in winter-derived plants) and GA and growth temperature (in summer-derived plants).

Results and Discussion

Winter-derived plants : herbage yield

The effect of GA and N combinations on yield of herbage (above 6 cm) are shown in Figure 1. There was a significant interaction between GA and N which changed during regrowth. After 3 weeks' regrowth, the effects of GA in increasing yield were greater at high N (i.e. 69.6%) than at low N (i.e. 15.2%) (main effect GA: $P<0.0001$; $F_{1,36}=21.8$; GA × N interaction: $P<0.001$; $F_{1,36}=13.4$). By week 4 (not shown; for full discussion see Parsons *et al.* 2012) of regrowth, however, GA had increased yield more at both low N (i.e. 25%) and high N (i.e. 38%) (GA: $P<0.01$; $F_{1,36}= 11.71$; GA × N: $P<0.05$; $F_{1,36}=4.66$).

Summer-derived plants : herbage yield

All measurements on summer-derived plants were made at high mineral N, but at two different temperature regimes. For both temperature regimes, after 3 weeks' regrowth, there was a small but statistically significant response to GA application (GA: $P<0.05$; $F_{1,156}= 5.27$; GA × T: N.S.).

Measurements of yield response to GA on a further subset of previously untreated plants were made (Parsons *et al.* 2012), using summer-derived plants, at both 12.5˚C (14 h day length) and 12.5˚C (8-h day length). These confirmed that the differences in response to GA in summer-derived plants compared with winter-derived plants were not due to differences in daylength during the period of measurement.

Winter- and summer-derived plants : roots

The biomass of roots of winter-derived plants showed major GA × N interactions that changed during regrowth (Figure 2). At low N, by 2 weeks of regrowth, adding GA resulted in decreased root mass; this can be seen by comparing the plus GA low N root mass with that of the minus GA low N control treatments which had been sustained for over 10 weeks previously (Figure 2) (GA = N.S.; GA × N $P<0.01$; $F_{1,36}=10.32$). After 3 weeks' regrowth, root growth was marginally increased by GA at low N (GA: $P<0.05$; $F_{1,36}=5.75$; GA × N $P<0.01$; $F_{1,36}=8.05$). By week 4, root growth at low N was significantly greater than in controls (GA: $P<0.01$; $F_{1,36}=9.42$; GA × N $P<0.05$; $F_{1,36}=5.02$). At high N, adding GA did not reduce root biomass.

In summer-derived plants at 3 weeks regrowth, GA had no significant effect on root mass (Figure 2).

Factors affecting capacity to respond to GA

Nitrogen supply

Our results suggest that the capacity to respond to GA, in producing more herbage, can depend on having an adequate supply of N. In this study, N supply (seen in winter-derived plants) had more effect in altering how long it took the plants to respond to GA, than whether they responded at all. That plants at low N supply responded at all to GA is noteworthy, as this suggests there has been a change in nitrogen use efficiency (Parsons *et al*. 2012). Our study showed a low response to GA in summer-derived plants, even at high N supply. This indicates that a low response to GA in summer in the field is unlikely to be due to a lack of N supply, alone.

Temperature

In this study we did not establish optimum temperatures for a response to GA, but we can suggest that differences in temperature in the field between early season (when GA is reported to be more effective) and mid-summer/autumn (when it is less effective) (see Leben *et al.* 1959; Williams & Arnold 1964) are unlikely to be due to the natural differences in temperature alone: our results indicate major differences in plants' capacity to respond to GA (compared with controls), even when the plants are measured at the same temperature (compare winter-derived plants with summer-derived plants when both are measured at 12.5°C).

Developmental state

The major difference in response to GA (compared with controls) between winter-derived and summer-derived plants in our controlled environment study suggests that seasonal changes in plant developmental state may have a major effect in the field on the capacity for the plants to respond to exogenous GA application.

These observations are consistent with those of previous studies of seasonal developmental changes in plant physiology and growth (Peacock 1975a,b; Parsons & Robson 1980, 1981a,b). In a field-based study, Peacock (1975a,b) found the rates of leaf extension, when plotted in relation to the mean temperatures in the region of the stem apex, were substantially different in spring and in autumn. He reported that leaf extension rates in spring (plants induced to flower) were greater than in autumn (vegetative) plants across a wide range of naturally occurring temperatures. Parsons & Robson (1980) plotted a time course of changes in leaf extension as the seasons progressed, by taking plants from the field at intervals and measuring their potential for leaf extension under controlled conditions, at 15°C. There were major seasonal differences in the potential for leaf extension (see solid symbols, Figure 3). It appears plants display their maximum potential for leaf extension (which is in spring/early summer) only after experiencing the pattern of environmental changes (light, daylength and temperature) over a previous autumn and winter. Plants growing in spring/early summer that have not had that experience show lower rates of extension (see open symbols Figure 3). In the context of GA, Figure 3 suggests there may be a greater gap in growth potential, which we propose GA may be "closing", in winter-derived plants than in summer-derived plants. Although Figure 3 relates to leaf extension and not dry weight (yield) gains, a very similar pattern of changes was observed in the photosynthetic capacity of leaves and assimilate partition to roots (Parsons & Robson 1981a,b) which lead to seasonal differences in yield.

Conclusions and Implications

Our results suggest that seasonal changes in the developmental status of plants of an important perennial grass species may have a major, possibly over-riding, effect on their potential to respond to GA.

Having an adequate supply of N at the time of GA application will be important to meet the N requirements for any growth response to GA, and this emphasises that GA application should not be seen as a substitute for N fertiliser inputs. However, the lack of response to GA in our studies in summer-derived plants, even at high N supply, suggests that adding extra N fertiliser in summer in the field is unlikely to increase the response to GA substantially. Seasonal differences in N supply and temperature will clearly create seasonal differences in growth rates, but these would be apparent in both controls and GA treated plants.

The effect of repeated applications of GA *per se* on the capacity to sustain a response to GA, remains an important topic for future research. Where a major response to GA was seen, in our winter-derived plants, it would appear that GA accelerates the production of leaves to an extent which initially draws heavily upon substrates (and so biomass) from roots. This was particularly evident at low N. In our study, the increased investment in leaves "paid off", as there was a subsequent increase in total plant biomass, and root mass not only recovered, but eventually increased, relative to controls. In practice, many factors could increase the risk of whether the plants ultimately benefit from having drawn on root mass. Any worsening of growing conditions, or a second defoliation early in regrowth, would clearly lead to adverse consequences, which would affect subsequent regrowth even without further GA application.

Plants can and do alter their potential for growth at different times of year. GA application is likely to be interacting with, or triggering part of, that natural process. This exposes new prospects for making sustained increases in plant growth, but only if the fundamental mechanisms of how plants control their response to environmental signals (e.g. temperature and soil N status) can be understood. The role of gibberellins (endogenous as well as externally applied) in changes in plant growth strategy, presents a new challenge for forage plant science.

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