Tactical use of gibberellic acid and nitrogen fertiliser to improve production of perennial ryegrass and white clover swards

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Abstract

The timing and frequency of exogenous gibberellic acid (GA) application was explored in a cut-and-carry small plot experiment using irrigated Canterbury swards. GA applications (8 g GA/ha/application) replaced urea applications (25 kg N/ha/application) over multiple harvests in late winter, early spring and autumn. Timing and number (1, 2 or 3 applications) of GA application occurred in combinations to test the effect of early (August and February), delayed (September and March) or late (October and April) application of GA. Annual herbage yield was 14.6, 18.3 and 18.3±0.40 t DM/ha/y for the three controls (0, 250 kg N/ha/y and 250 kg N/ha/y + 80g GA/ha/y respectively). Clover content varied with treatment and time of year and was more abundant in low N treatments. There was a positive relationship between N fertiliser rate and annual herbage yield. There was no relationship between increasing GA rate and annual yield, though there was evidence of annual yield improvements, associated with frequent GA application under low (100 kg N/ha/y) or moderate (150 kg N/ha/y) N fertiliser. In late winter, delaying a single application of GA into spring improved N use efficiency (P<0.01). Tactical use of GA, in place of N fertiliser, can be used to maintain herbage yield.

Keywords: gibberellin, *Lolium perenne, Trifolium repens*, GA_3, plant hormone, nitrogen response

Introduction

Achieving high levels of dry matter (DM) production on dairy farms in New Zealand has, over the last 30 years, relied on increasing rates of nitrogen (N)-based fertiliser to meet feed supply targets (Fertiliser Association, 2018). Nitrogen fertiliser has played an important role in improving pasture production, and, in particular, improving milk yield through extending the growing season and the number of days in milk (McGrath et al., 1998). However, excessive use of N inputs is linked with increased pollution from nitrous oxide and nitrate leaching (Ledgard et al., 1997). To reduce the use of N fertiliser, government regulations has limited annual applications to a maximum of 190 kg N/ha/year.

Alternative pasture promotants, such as the naturally occurring growth hormone gibberellic acid (GA), can be used to manage feed supply under capped synthetic N fertiliser use. Gibberellic acid stimulates plant growth by switching off dormancy and mobilising plant reserves resulting in stem and leaf elongation (Sun 2008). When applied at low rates GA has the potential to produce a cost-effective increase in DM production in autumn, late winter or spring (Matthews et al., 2009, Edmeades 2009, Ball et al., 2012, Parsons et al., 2013, van Rossum et al., 2013, Zaman et al., 2014).

A review of GA by Matthew et al., (2009), which compared results from a variety of pasture species and environments, noted that responses to various GA application rates ranged from 60-1300 kg DM/ha compared with untreated controls without N fertiliser. More recent studies, based in New Zealand using modest GA rates, showed yield increases of 126 to 1199 kg DM/ha compared to untreated controls without N fertiliser (Zaman et al., 2014). In spite of being cost effective to use (Matthew et al., 2009), adoption is not widespread, which might be associated with known side effects relating to a yield depression following the first or second regrowth after application (Matthew et al., 2009, Bryant et al., 2016). Such yield depression may be due to reduced access to nutrients through prior depletion of root reserves and subsequent partitioning of nutrients below ground (Ball et al., 2012). While there is evidence that the timing of exogenous GA relative to supply of nutrients such as N may influence the short-term response to GA (Zaman et al., 2014), there is limited information regarding the long-term impact of GA use. Given that the initial and secondary herbage response to GA appears to be closely linked to soil N fertility (Ball et al., 2012) there is value in exploring the impact of timing and frequency of GA application on the long-term productivity of perennial ryegrass-based pastures. The purpose of this study was to compare the effect of timing and frequency of exogenous GA, as a replacement for N fertiliser, on seasonal and annual DM production. Using a series of GA x N treatments, three null hypotheses were tested:

1. Replacing N fertiliser with exogenous GA will have no effect on DM yield

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2. The timing of GA application in spring and autumn will not affect DM yield
3. Frequent applications of GA will not affect DM yield

Materials and methods

A plot study was conducted between 2 August 2012 and 16 June 2013 at the Lincoln University Research Dairy Farm, Canterbury, New Zealand (43°38'S, 172°27 E, 17 m.a.s.l.). The 0.1 ha experimental site was situated under a lateral irrigator on a Wakanui silt loam soil with a pH of 5.7 and Olsen P of 20 and MAF quick test potassium of 4. The area had previously been established following full cultivation in April 2011. At that time, the existing perennial ryegrass pasture was sprayed with glyphosate, ploughed, harrowed and *Lolium perenne*, (cv Trojan, AR37 endophyte, heading date + 16 days) and white clover (*Trifolium repens*, cv Weka) was sown at a respective rate of 18 and 4 kg seed/ha using a coulter drill. Following establishment, herbage mass was controlled by rotational grazing with dairy cows.

Experimental design

The experiment was a randomised complete block design with ten treatments (plot size = 7 x 2 m) in four blocks. Treatments were selected to investigate the impact of timing and frequency of GA as a replacement for N fertiliser. The ten treatments include three controls: 1. N control (250 N, 0 GA), 2. positive control (untreated, 0 N, 0 GA), and 3. negative control (250 N, 80 GA), and seven GA regimes including single GA applied early (E), delayed (D), late (L) or a combination of ED, EL, DL and EDL in late winter, early spring, late summer and autumn. For details of timing, number and application rates see Table 1, with winter harvests occurring on 14 May and 16 June. For convenience the regrowth period from the first application in August to the third harvest in October was referred to as the spring response and similarly February to May was referred to as the autumn response.

Prior to the first application of N fertiliser or GA in August, the experimental area was mown to a height of 4.5 cm. The N fertiliser was applied by hand at a rate of 25 kg N/ha as urea granules (46% N). The GA was applied by diluting 1.6g ProGibb® SG in 16 l of water and applied, according to manufacturer's directions (Nufarm Ltd, Ackland, NZ), at a rate of 8 g GA/ha as ProGibb® SG (40% GA) with non-ionic surfactant, Contact™ (0.25 ml/l). The GA was applied within 72 h of defoliation using a knap sack and calibrated walking speed to ensure each plot received the correct rate of product (equivalent to 200 l water/ha).

All measurements followed an average regrowth interval of 31±8 d and an average of 28±7.5 d from time of application to harvest. The actual regrowth interval was weather dependant and based on decision rules related to minimum two leaf appearance, averaged across all treatments. Following each re-growth period (n=10), the plots were harvested using a rotary mower set to a cutting height of 4.5 cm. The total catcher fresh weight was recorded and a subsample weighed, dried to a constant weight and reweighed for the determination of dry matter content and yield estimation. Prior to each harvest in spring and autumn, snip samples were harvested to 4.5 cm and a representative sample was sorted into sown and weed species and dried to determine botanical content. Within three days of harvesting, the N and GA treatments were applied to designated. To minimise variation caused by animal excreta the plots were not grazed during the study period and no fertiliser other than urea was applied. The area was irrigated during the study to maintain pasture growth over the entire period.

Table 1 Nitrogen (N) and gibberellic acid (GA) treatments and application dates in 2012

Treatment	2-Aug	4-Sep	5-Oct	31-Oct	26-Nov	18-Dec	21-Jan	11-Feb	14-Mar	12-Apr	kg N/ha/y	g GA/ha/y
Untreated	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0	0
N fertiliser	N	N	N	N	N	N	N	N	N	N	250	0
GA+N fertiliser	GA+N	GA+N	GA+N	GA+N	GA+N	GA+N	GA+N	GA+N	GA+N	GA+N	250	80
Early GA	GA	N	N	N	N	N	N	GA	N	N	200	16
Delayed GA	N	GA	N	N	N	N	N	N	GA	N	200	16
Late GA	N	N	GA	N	N	N	N	N	N	GA	200	16
EL	GA	N	GA	N	N	N	N	GA	N	GA	150	32
ED	GA	GA	N	N	N	N	N	GA	GA	N	150	32
DL	N	GA	GA	N	N	N	N	N	GA	GA	150	32
EDL	GA	GA	GA	N	N	N	N	GA	GA	GA	100	48

Where E=early, D=delayed, L=late applications.

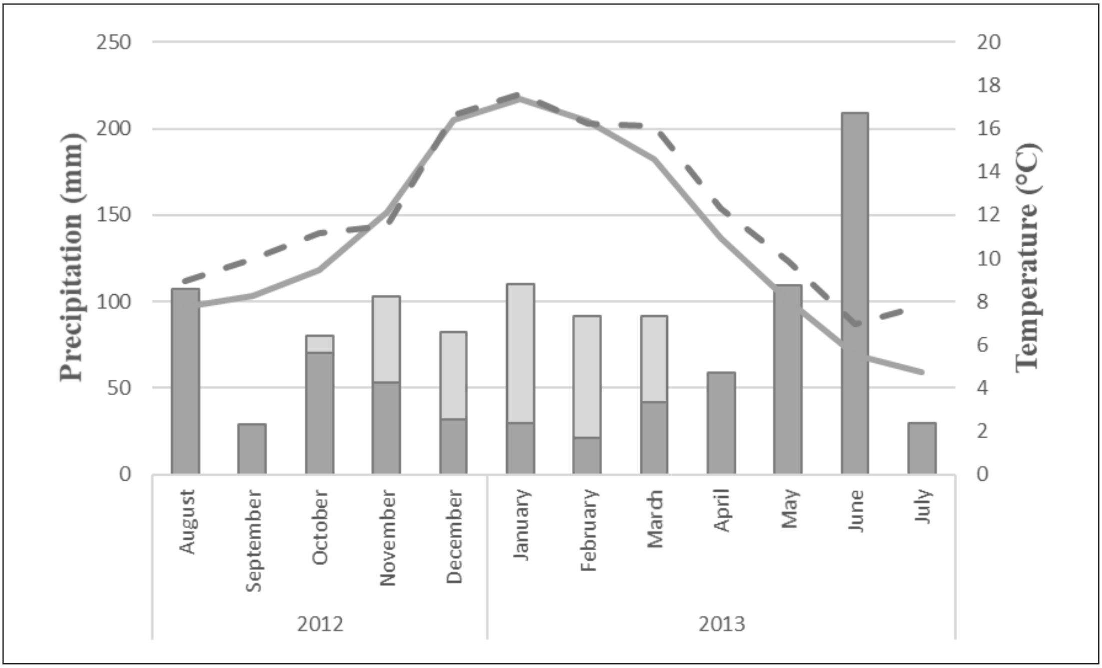

Figure 1 Average air temperature (solid line), average 10cm soil temperature (dashed line) and monthly irrigation (light shaded column) and rainfall (dark shaded column)at the Broadfields weather station near the Lincoln University Research Dairy Farm between August 2012 and July 2013.

Statistical analysis

Means for annual and seasonal yield and N response were compared using the general linear model procedure in Genstat (V19.1, VSN International Ltd). Fertiliser treatment was included as a fixed term in the model and block as blocking term. Repeated measures were used to analyse botanical content over the three sampling dates each in spring and autumn. Linear regression was performed to determine the relationship between rate of N fertiliser or GA and annual herbage yield. Contrasts were carried out to compare the effect of a single (E+D+L) versus double (ED+EL+DL), and or early (E + ED) versus late (L + DL), application of GA. Fishers protected LSD was used to separate means for each treatment at the 5% significance level.

Results

Total rainfall over the 12 month observation period was 790 mm, with the largest rainfall event occurring at the end of the measurement period in June 2013 (Figure 1). Applications of GA occurred when soil temperatures were above 7.5°C. Irrigation applied 310 mm between October and March at a rate of 10mm per application (Figure 1) to give a total precipitation of 1100 mm.

Compared to the 0 control (14.6 t DM/ha/y), annual herbage yield was 18.3±0.40 t DM/ha/y at 250 kg N/ha/y with or without GA (Table 2). Annual herbage yield declined by 4% following replacement of two N applications (-50 kg N/ha/y) with GA and by 8% when four applications of N (-100 kg N/ha/y) were replaced by GA. Regression analysis of N rate and yield across all treatments revealed a positive linear relationship (kg DM/ha=14794 (SE 422) + 14.2 (SE 2.19) x kg N/ha/y, $P \leq 0.001$). In contrast with N fertiliser there was no relationship between GA application (between 0 and 48 g/ha) and yield (kg DM/ha=16771 (SE 373) + 13.8 (SE 11.1) x g GA/ha/y, $P=0.22$).

Interactions between harvest date and treatment showed variation in clover in response to treatment (Figure 2a). Average clover content was higher in spring than in autumn (30.2 ±1.5 % *vs*. 38.1±1.1 %, $P<0.05$). During the spring period, white clover content increased from 23 to 37±1.5% over the three harvests. Clover content in spring ranged from 23% in the treatments receiving 250 kg N/ha to 45% in the 0 control ($P<0.001$). Despite differences between treatments for clover content, the total cumulative yield of clover (948±126 kg DM/ha) did not differ between treatments (Figure 2b). Differences in total DM yield were driven by variation in yield of ryegrass (Figure 2c) which was greatest ($P<0.05$) for the GA+N treatment.

An interaction between harvest date and treatment ($P<0.05$, Table 2) revealed that in spring, delayed or late GA application resulted in greater herbage production than applying GA early. Applying N early and delaying GA application also improved the N response in spring,

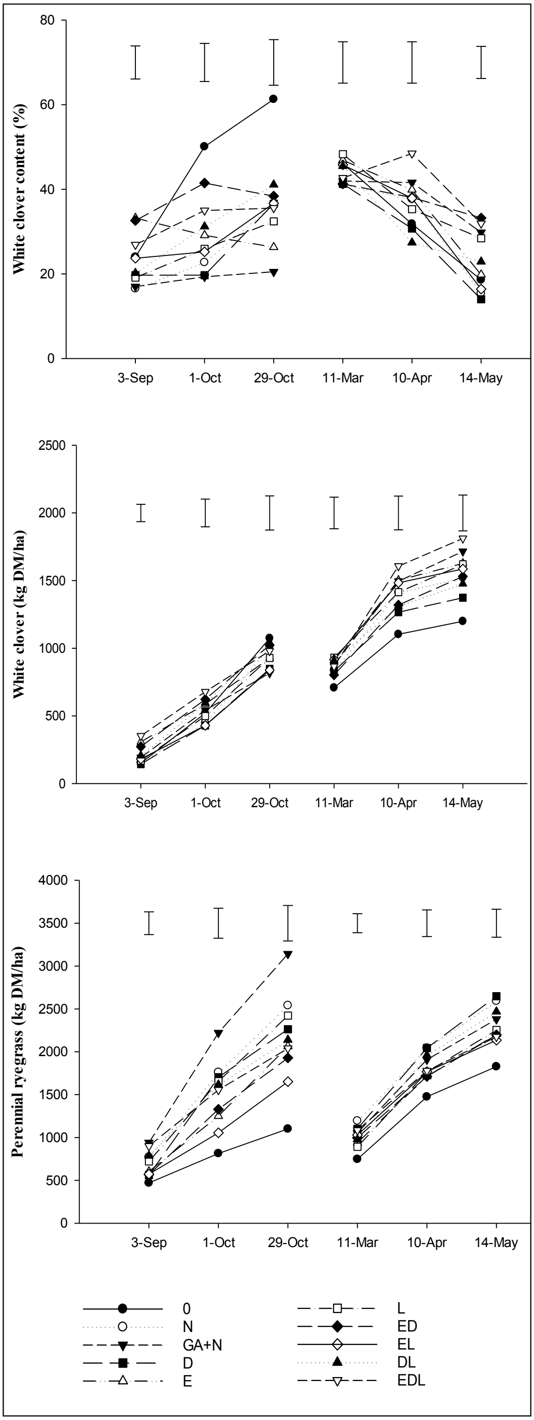

Figure 2 Herbage content (A) and accumulated yield (B) of white clover and perennial ryegrass (C) in spring and autumn following application of nitrogen (N), early (E), delayed (D) or late (L) gibberellic acid (GA). NB: see Table 1 for full description of treatments. Bars indicate standard error of the mean.

but not in autumn. The timing of GA application had carry-over effects on winter growth with improved pasture production from late GA applications. Interestingly, the EDL treatment, which received three consecutive GA applications had significantly greater spring DM yield compared with the 0 control, despite neither treatment receiving N fertiliser during this period (Table 2, $P<0.05$).

Although replacing a single application of N in both spring and autumn resulted in a 5% reduction in herbage yield this reduction was not statistically detectable. Generally, there was no effect of frequency of GA (one or two applications) on seasonal yield, with the exception of winter. When comparing N fertiliser with GA+N fertiliser, results showed that there was no yield reduction when using multiple applications of GA with N fertiliser. In both spring and autumn, the N response increased when N was swapped for GA twice rather than once, though over the whole season there was no significant effect of timing or frequency of GA on N response.

Discussion

Nitrogen versus gibberellic acid

The results demonstrated a positive relationship between N fertiliser and annual herbage accumulation. In this study, 250 kg N/ha/y resulted in an additional 3.7 t DM/ha/y compared with the untreated control and an annual N response of 14.8 kg DM/kg N under cut and carry conditions. The greater N response in spring compared with autumn in the current study is consistent with previous observations (O'Connor and Gregg 1971; Sun et al., 2008) and the annual response of 14.8 kg DM/kg N observed here is regarded as a good response (Sun et al., 2008). The response of pasture species such as perennial ryegrass to N fertiliser is well documented, with rapid uptake and assimilation of N triggering leaf expansion, tiller initiation and auxiliary bud outgrowth (Harris et al., 1996, Sun et al., 2008).

While N provides the resources for the synthesis of new tissues to increase yield, GA acts by reallocating those resources. For a single regrowth, exogenous GA is used to remobilise carbohydrate from crown and roots into leaf and stem elongation, driving yield through increased tiller mass (Matthew et al., 2009). However, that mobilisation can lead to subsequent nutrient deficits and, over a several weeks, the partitioning of assimilates moves resources back to those depleted areas. This was observed by Ball et al. (2012) where they demonstrated, under N limiting soil conditions, increased below ground partitioning of carbohydrates in GA-treated ryegrass. This switch from above to below ground partitioning is also evident in yield reductions observed in many trials following the first GA application and likely explains the lack of relationship between GA rate and annual yield found in the current study.

Timing of gibberellic acid

Although no statistical differences in annual yield was detected when GA replaced N, the results of this study highlight the value of timing of key elements in tactical pasture management. Tactical use of N and GA resulted in significant changes in yield in all seasons apart from summer. Parsons et al. (2013) showed that summer derived perennial ryegrass tillers were far less responsive to exogenous GA than winter-derived tillers. Similarly, response to N is summer is limited by development of reproductive tissues (Sun et al*.,* 2008), consequently the differences observed in the present study occurred during vegetative growth stages.

Most of the variation in yield occurred in late winter and early spring when ryegrass was dominant and responsive to N fertiliser (Figure 2). Use of early N fertiliser in late winter presents an opportunity to establish tiller populations providing the growing points for future yields. The tillering and promotion of auxillary bud outgrowth is influenced by the hormone cytokinin, which is dependent on the N status of the plant (Roche et al*.,* 2017). In contrast, GA suppresses outgrowth of auxillary buds (Zhaung et al*.,* 2019), resulting in more resources going into existing tillers to stimulate growth and drive the observed yield increase. Thus, if GA is applied in September or October, after N fertiliser, vernalised tillers respond positively to GA and an increased tiller population continue to develop under conditions of improved soil N.

Table 2 Effect of replacing applications of N fertiliser (25 kg N/ha/application) with gibberellic acid (GA, 8 g/ha/application) on pasture dry matter production (t DM/ha) and response to N (kg DM/kg N).

		2 Aug – 29 Oct		30 Oct – 10 Feb		11 Feb – 14 May		15 May – 6 Aug	Annual	
Treatment	**No. GA**	**Yield**	**Response**	**Yield**	**Response**	**Yield**	**Response**	**Yield**	**Yield**	**Response**
0 control		2.26^{d}	-	8.28	-	3.25^{c}	-	0.82^{g}	14.61^{e}	-
N control		3.70^{ab}	19.2^{c}	9.20	9.1	4.24^{ab}	13.2^{d}	1.19^{bcd}	18.32^{a}	14.8
N + GA		4.14^{a}	25.2^{bc}	8.56	2.8	4.37^{a}	15.0^{cd}	1.26^{abc}	18.34^{a}	14.9
Early GA	1	3.13^{bc}	17.5^{c}	8.93	6.5	4.00^{ab}	15.2^{cd}	1.20^{bcd}	17.27^{abcd}	13.3
Delayed GA	1	3.51^{ab}	25.2^{bc}	8.90	6.2	4.18^{ab}	18.7^{cd}	1.12^{cde}	17.72^{abc}	15.5
Late GA	1	3.49^{ab}	24.8^{bc}	9.04	7.6	3.96^{ab}	14.2^{cd}	1.40^{a}	17.89^{ab}	16.4
ED	2	2.72^{cd}	18.8^{c}	9.02	7.4	3.91^{b}	26.4^{bcd}	0.90^{fg}	16.55^{d}	12.9
EL	2	3.12^{bc}	34.6^{ab}	8.41	1.2	4.06^{ab}	32.3^{ab}	1.28^{ab}	16.86^{bcd}	15.0
DL	2	3.33^{bc}	42.3^{a}	8.59	3.1	4.16^{ab}	36.6^{a}	1.04^{ef}	17.12^{bcd}	16.7
EDL	3	3.10^{bc}	-	8.32	0.4	4.18^{ab}	-	1.08^{de}	16.68^{cd}	20.7
SEM		0.248	3.89	0.284	3.01	0.145	4.19	0.070	0.397	2.99
P Value		0.001	0.002	0.26	0.42	0.001	0.003	<0.001	<0.001	0.78
Contrasts*										
Early *vs.* Late		0.06	<.001	0.57	0.60	0.48	0.28	0.002	0.15	0.26
One *vs.* two		0.12	0.007	0.24	0.27	0.94	<.001	<.001	0.02	0.94

Treatments were untreated pasture (0 control); pasture treated with N fertiliser in spring, summer and autumn without (N control), or with gibberellic acid (N + GA); single applications of GA applied in place of N fertiliser in spring and autumn as either early (E), delayed (D), or late (L), or two (EL, ED, DL) or three applications of gibberellic acid (EDL).

*Where contrasts for early versus late = E+ED *vs.* L+DL and one *vs.* two =E+D+L *vs.* ED+EL+DL

In contrast with spring, N was unlikely to be limiting pasture growth in autumn, with additional N from summer fertiliser applications. The current results showed that, in autumn, GA alone increased yield compared with the untreated control and achieved similar yield as using N fertiliser. There appeared to be some carry over effects of timing of GA on winter growth. Swapping N for GA late into autumn improved winter production compared with early use of GA (Table 2). The reason for the improved winter growth was not clear, as the yield effects occurred in the second and third regrowth after application. As daylength declines the effects of exogenous GA in barley has shown to be prolonged (Cottrell et al., 1982) and improved yield due to GA may be associated with improved light interception over winter. In previous studies GA application increased specific leaf area (Djikstra et al., 1990), which enhanced interception of photosynthetically active radiation. More research is required to confirm this hypothesis.

Early use of N fertiliser (late winter and late summer) was more effective for improving yields than later applications. The effect of timing of GA for tactical management does present some opportunities to reduce N fertiliser use with minimal reductions in pasture yield. For example, applying N alone at 250 kg N/ha/y, the Lincoln site grew an additional 3.70 t DM/ha over the untreated control, giving a response of 14.8 kg DM/kg N. Assuming a linear response to N, by reducing inputs from 250 to 200 kg N/ha/y this could be expected to reduce annual yield by 4% without including GA (Table 3). However, depending on the timing of the application, when N is swapped for GA annual yield was reduced by either 6% (early GA) or reduced by only 2% (delayed/late GA).

Frequency of gibberellic acid applications

This experiment hypothesised that one-off exogenous GA applications in spring and autumn could be used tactically to reduce N inputs and maintain pasture production. Replacement of more than 50 kg N/ha/y with GA led to lower annual pasture yields (Table 2), not because of the increased GA per se, rather the diminishing availability of N. Perennial ryegrass was not expected to continuously respond to multiple applications of exogenously applied GA_3 as changes in sensitivity of pasture to exogenous GA occur throughout the season. Early research where yield reductions were seen used either high rates of GA (exhausting carbohydrate mobilisation) or applied it in winter and/or without mineral N (Matthew et al., 2009). The current results suggested that yield reductions from GA use could be avoided with either co-application of N or delaying GA application until the second regrowth and applying N fertiliser in the first regrowth.

While perennial ryegrass may become unresponsive to GA, this may not be the case for white clover. Based on prediction equations, applying 100 kg N/ha/y should have resulted in 16.3 t DM/ha/y, but instead, in combination with 72 g GA/ha produced 16.9 t DM/ha/y (Table 3). The treatments with the largest number of GA applications (N+GA and EDL) had high clover yield, particularly in autumn, which may have offset expected yield loss. Positive response of white clover dry matter yield to exogenous GA, has previously been reported (Matthew et al., 2009, Bryant et al., 2013), with no negative effect on nodulation (Fletcher et al., 1958). From a practical perspective for farmers using very low annual rates of N fertiliser, regular use of GA might be a useful tool to encourage clover and maximise pasture yield.

Table 3 Difference between observed and predicted (14.8 x kg N fertiliser) annual pasture production (kg DM/ha) above baseline (14.8 t DM/ha/y) if GA was not used.

	N rate	Predicted	observed	difference	% change
N control	250	3700	3705	5	0%
N + GA	250	3700	3724	24	1%
Early GA	200	2960	2657	-303	-10%
Delayed GA	200	2960	3107	147	5%
Late GA	200	2960	3279	319	11%
ED	150	2220	1939	-281	-13%
EL	150	2220	2250	30	1%
DL	150	2220	2509	289	13%
EDL	100	1480	2068	588	40%

Where: Treatments were pasture treated with N fertiliser in spring, summer and autumn without (N control), or with gibberellic acid (N + GA); single applications of GA applied in place of N fertiliser in spring and autumn as either early (E), delayed (D), or late (L), or two (EL, ED, DL) or three applications of gibberellic acid (EDL).

Conclusion

Under regulatory N fertiliser caps, the replacement of autumn applied N with GA represents a valuable pathway to maintain end of season pasture production and reduce environmental impacts of farming. When applied alone in place of N, delaying GA application until after the first or second N fertiliser application can improve dry matter yield. This is the first research to report the effects of single or multiple applications of GA on annual above ground pasture production. These results showed that N fertiliser is far more important in driving herbage yield than the plant growth regulator GA. Due to the high potential for growth in spring, GA used in combination with N fertiliser can improve production. The results indicated that GA can be used effectively in autumn to maintain herbage yield in replacement of N. Therefore, under regulatory N fertiliser caps, the replacement of autumn applied N with GA represents a valuable pathway to maintain end of season pasture production and reduce environmental impacts from farming.

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