Agronomic assessment of gibberellic acid and cytokinin plant growth regulators with nitrogen fertiliser application for increasing dry matter production and reducing the environmental footprint

A. GHANI[1], S. LEDGARD[1], J. WYATT[1], W. CATTO[2]
[1] *AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand*
[2] *Ballance Agri-Nutrients Limited, Tauranga, New Zealand*
anwar.ghani@agresearch.co.nz

Abstract
Plant growth regulators (PGRs) are increasingly being used in agriculture for a variety of purposes. In pasture systems, the use of PGRs is relatively new and the effects of its application on plant productivity and physiology are poorly understood. A mowing trial was established in Waikato in early spring 2012. Key objectives from this study were: to determine effects of gibberellic acid (GA_3) and cytokinin (CPPU) application with and without nitrogen (N) fertiliser application on pasture production, N concentration (%N), root biomass and potential reduction in N leaching from grazed pastures. Treatments (with five replicates) were: control, urea (40 kg N/ha), GA_3 at two rates +/- urea, and CPPU at three rates +/- urea. Implications for grazed pasture and N leaching were modelled for a case study dairy farm. Application of GA_3 alone showed a significant ($P<0.05$) rapid increase in dry matter (DM) production compared with the control between 5 and 29 days after application. The DM yields from GA_3 alone were similar to those with urea-N alone and the effects of applying GA_3 and urea-N together were additive. Application of CPPU showed no significant effect on pasture production. There were no treatment effects from either PGR on root biomass. The %N in herbage was significantly lower in the GA_3 treated plots than the control, which would reduce urinary-N excretion under grazing. Preliminary modelling of data for a grazed pasture showed a potential reduction in annual urine-N leaching of 4–29%.

Keywords: Gibberellic acid (GA_3), cytokinin (CPPU), dry matter yield, total N, root biomass

Introduction
Plant hormones are a group of naturally occurring organic compounds which influence a number of physiological processes in plants (Davies 1995). Hence, plant hormones or plant growth regulators (PGRs) are important to internally coordinate the growth and development of different plant organs and as a chemical messenger. Gibberellins are plant hormones that activate dormant enzyme systems. Applied to pasture, they can stimulate out-of-season growth or accelerate growth through reserve nutrient mobilisation, leaf and stem elongation, and promotion of flowering (Matthew *et al*. 2009).

Cytokinins are a group of chemicals that influence cell division and shoot formation. They also help delay the senescence or aging of tissues, are responsible for mediating auxin transport throughout the plant, and affect internodal length and leaf growth (Davies 1995). There is very little published data on the use of cytokinins in pastoral systems (Matthew *et al*. 2009). The purpose of this research was to understand the potential benefits of two PGRs, namely gibberellin and cytokinin, for enhancing the pasture response to N fertiliser in early spring. Key research questions were: i. Can PGRs enhance the pasture response from N fertiliser at times when feed demand is high but natural plant growth is low e.g. in early spring? and ii. Do PGRs consistently lower the N concentration in herbage, and what is the potential to reduce the environmental risk by reducing N leaching from their use on grazed pastures?

Materials and Methods
A small plot field trial was established on long-term pasture located on a moderately well-drained volcanic soil at the Ruakura Research Centre, Hamilton. The permanent pasture was predominantly perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). The site had previously been grazed by dairy cattle but had been excluded from grazing since April 2012, with the pasture being mown and removed. Prior to application of treatments, a covariate cut was taken and soil samples were collected for testing soil fertility. Based on that analysis, 20 kg P/ha, 23 kg S/ha and 40 kg K/ha were applied to the trial site.

Twelve treatments (Table 1) with five replicates were applied in early September. Gibberellic acid (GA_3), cytokinin (CCPU) and urea-only sprays were applied at a rate of 800 litres/ha with a non-ionic surface surfactant (trade name: Contact, Nufarm) added at 1.0 ml/litre and the same volume of water with the same concentration of surface surfactant was sprayed on the control.

Each plot was divided into four 0.5 m wide subplots. Herbage was cut separately from individual subplots at 5, 10, 20 and 29 days after treatments were applied and from all plots at 29, 67 and 97 days. Herbage samples were dried for 72 hours at 65°C and dry matter (DM) yield was measured. Dried herbage samples from day 20 were analysed for total N by Kjeldahl digestion and total potassium (K) and total phosphorus (P) by acid digestion and ICPOES determination (Anderson 1996). Seven soil cores (2.5 cm diameter) were taken from each plot, to depths of 0–7.5 cm and 7.5–15 cm, on days 29 and 97 after treatment application. These cores were washed and the sieved root biomass material was dried at 65°C for 72 hrs for DM determination.

Statistical analysis

Analysis of variance of all pasture data for DM, N, P and K parameters was carried out using the statistical software package GenStat, 13th Edition. All DM yield analyses were adjusted for covariate yields.

Modelling of effects on N leaching

An example Waikato dairy farm was used to model the effects of changes in pasture %N from GA_3 use on urine-N excretion and N leaching. The model analyses examined the effects of seven treatments (GA_3 applied at 20 g/ha):

1. GA_3 applied in April-only with a reduction in pasture N concentration from 3.9% to 3.6% over a 1-month period after application,
2. GA_3 applied in April-only with a reduction in pasture N concentration from 3.9% to 3.4% over a 1-month period after application,
3. GA_3 applied in April-only with a reduction in pasture N concentration from 3.9% to 3.2% over a 1-month period after application,
4. GA_3 applied three times between April and August with a reduction in pasture N concentration from 3.9% to 3.6% during the April-August period (i.e. for 5 months),
5. GA_3 applied 3 times between April and August with a reduction in pasture N concentration from 3.9% to 3.4% during the April-August period (i.e. for 5 months),
6. GA_3 applied 3 times between April and August with a reduction in pasture N concentration from 3.9% to 3.2% during the April-August period (i.e. for 5 months),
7. Same as 6 except that it was assumed that GA_3 use in April and August replaced N fertiliser (30 kg N/ha) at these times.

The reductions in pasture N concentration by 0.3–0.7% reflect the range in N reductions measured in the field trials (Zaman *et al.* 2014; this study).

The Waikato dairy farm was on volcanic soil with 3 cows/ha producing 1000 kg milk solids/ha/year. It was assumed that N fertiliser was applied in split dressings (between August and March) at 200 kg N/ha/year (although this had little effect on relative treatment effects because of the fixed pasture N concentrations). It was assumed that farm dairy effluent was treated using a two-pond system (for simplicity of modelling).

These farm systems were analysed using a beta version of OVERSEER® that was modified to enable monthly pasture N concentrations to be fixed. The pasture N intake by cows was calculated as well as the total amount of N excreted (after accounting for N removal in product) and the amount of N excreted in urine (using the equation in OVERSEER® for excreta-N partitioning between urine and dung; Ledgard *et al.* 2003).

Table 1 Details of treatments of gibberellic acid (GA_3), cytokinin (CCPU) and N fertiliser applied to the field mowing trial.

Treatments	GA_3 (g/ha)	CCPU (g/ha)	Urea (kg N/ha)
1. Control	0	0	0
2. 20 GA_3	20	0	0
3. 20 GA_3 with urea	20	0	40
4. 30 GA_3	30	0	0
5. 30 GA_3 with urea	30	0	40
6. 20 CCPU	0	20	0
7. 30 CCPU	0	30	0
8. 50 CCPU	0	50	0
9. 20 CCPU with urea	0	20	40
10. 30 CCPU with urea	0	30	40
11. 50 CCPU with urea	0	50	40
12. Urea only	0	0	40

Results

Gibberellic acid (GA_3)

The cumulative DM yields after treatment application are presented in Figure 1a. The effect of GA_3 application on DM yield was apparent immediately, showing significantly ($P<0.05$) greater yields than the control at 5 and 10 days after application. At 29 days after treatment application, the GA_3 alone treatments at both rates (20 and 30 g/ha) produced significantly ($P<0.001$) higher DM yields than the control i.e. 1553 and 1747 kg DM/ha compared to 1040 kg DM/ha. This represented a response of 50 and 68%, respectively, but there was no significant difference between the two GA_3 application rates on DM yield. At 29 days after treatment application, the GA_3 + urea treatments (with GA_3 at 20 or 30 g/ha) produced the highest DM

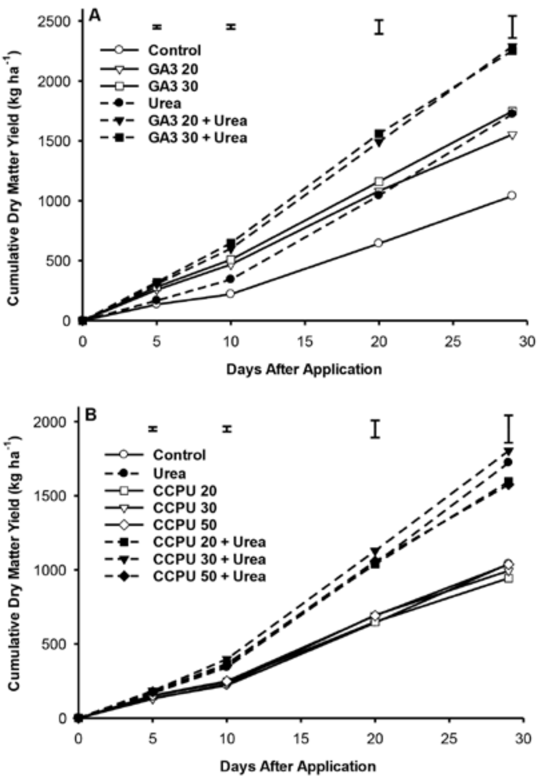

Figure 1 Cumulative dry matter yields (kg ha^{-1}) from (A) control, GA_3 and GA_3 + urea treated plots urea, and (B) the CCPU treated plots at 5, 10, 20, 29 days after treatment application. Error bars represent the least significant difference (LSD *P*<0.05) between treatments.

yields compared to any other treatments (Figure 1a), averaging 119% more than the control, 47 and 29% more than GA_3 alone at 20 and 30 g/ha, and 32% more than the urea-N only treatment, respectively. These results clearly show the additive effects of applying GA_3 and urea-N together.

The urea alone and GA_3 alone treatments produced a similar amount of DM at 29 days after treatment application. However, there was a lag effect of between 5 and 10 days, with the more rapid response from the GA_3 treatments (Figure 1a), and with the initial DM yields from urea-alone being significantly (P<0.05) lower than the GA_3 alone treatments.

There was no significant effect of GA_3 treatments alone or with N on DM yields from cuts done 67 and 97 days after treatment applications (data not presented). Total cumulative DM yields from three cuts (i.e. 29, 67 and 97 days after the treatment application) in control, GA_3 (mean of both rates), urea and GA_3+N (mean of both rates of GA_3+N) treatments were 4300, 4400, 4450 and 4800 kg/ha respectively (LSD at P<0.05 428 kg/ha).

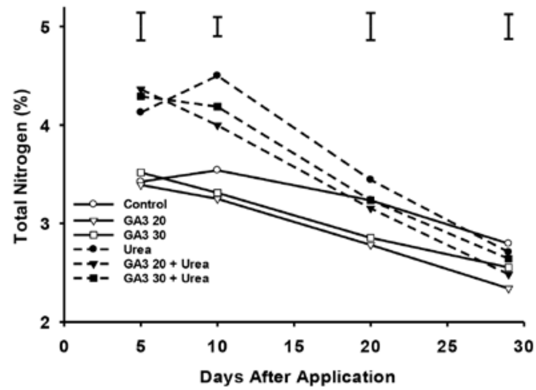

Figure 2. Herbage total nitrogen concentration (%) for the control, urea only and GA_3 treated plots at 5, 10, 20, and 29 days after treatment application. Error bars represent the least significant difference (LSD *P*<0.05) between treatments.

Cytokinin (CPPU)

The DM yield results from the CPPU, CPPU+Urea treatments are presented in Figure 1b. All three rates (20, 30 and 50 g ha^{-1}) of the CCPU only treatments had no effect on DM yield. Similarly, there was no significant difference in yields between the urea only treatment and the CCPU + urea treatments, showing that the DM responses in these treatments were solely due to N from the urea fertiliser. Since there was no agronomic advantage of applying CPPU, most of the results and discussion henceforth is focussed on GA_3 treatments.

Nitrogen concentration in herbage

The application of urea-N either on its own or with the GA_3 treatments significantly increased (P<0.05) herbage N concentration at 5 and 10 days after treatment application (Figure 2). These values exceeded 4.2% and peaked at 4.5% compared to 3.4% total N in the control. Application of GA_3 alone significantly (P<0.05) lowered the %total N in herbage from 5 to 29 days after treatment application, declining to 2.3 and 2.5% for the 20 and 30 g GA_3/ha treatments, respectively, at day 29 (Figure 2). On day 29 the GA_3 20 g/ha + urea treatment had a significantly (P<0.05) lower total N concentration than the control. Application of CCPU had no significant effect on the herbage N concentration (data not presented).

Phosphorus and potassium concentrations in herbage

Concentrations of P and K in the herbage DM at 20 days after treatment application showed no significant difference between the control, urea only, GA_3 only, GA_3 + urea treatments. Potassium concentrations ranged from 3.1 to 3.6% and P concentrations from 0.47 to 0.53%.

Root biomass

Root biomass assessed at days 29 and 97 after treatment application showed that most roots (c. 80%) were in the top half of the 0–15 cm depth and showed no significant effect of treatments. However, at day 97, several treatments showed slightly lower ($P<0.05$) root biomass in the lower 7.5–15 cm depth, although there was no obvious trend due to treatment or PGR rate (data not presented).

Modelling of effects on N leaching

The results from analyses for the Waikato dairy farm showed reduced pasture-N intake by cows from the GA_3 treatments by up to 7.6% due to the lower pasture N concentrations (Table 2). Output of N in the product was assumed to be the same across all GA_3 treatments, so that there was a slightly larger GA_3-induced reduction in total N excreted by up to 9.5%. The proportion of the N excreted in urine relative to dung decreases with decreasing dietary N concentration and therefore the model predicted a larger overall reduction in the amount of N excreted in urine of up to 13.3%. The latter applied to the treatment 6 where the GA_3 effect was assumed to last for 5 months and during that period (April-August) there was a reduction in urine-N excreted of up to 31%. However, while OVERSEER® predicted a relatively higher proportion of the urine-N lost by leaching during this late-autumn to winter period (27–32% for April-July and 7% for August) compared to the spring to early-autumn period (0–20%), it also recognised that the pasture intake by cows is less during this late-season and non-lactating period compared to that during the main lactation period. The net effect of this is that urine-N leaching between April and August (i.e. over 42% of the year) represented about 54% of the annual urine-N leaching. Thus, the reduction in N leaching from urine varied between 4 and 14%. Further analysis for scenario 7, assuming GA_3 effectiveness over 5 months with a pasture N concentration of 3.2% N but with removal of N fertiliser application in April and August (assuming the same DM production was achieved using GA_3 instead of the fertiliser-N), resulted in a decrease in urine-N leaching of 29%. The beta version of OVERSEER® used for these analyses requires wider testing, particularly in relation to N leaching from the non-urine-N sources which were higher for scenarios with reduced pasture %N so that overall apparent reductions in N leaching were less than shown in Table 2. Thus, the estimates in Table 2 represent a potential maximum reduction in N leaching.

Discussion

The rapid DM responses within 5–29 days of exogenous application of GA_3 in our study were of a similar magnitude to those which have been observed in other recent studies (e.g. Zaman *et al.* 2014; Van Rossum *et al.* 2013; Edmeades & McBride 2012). While there was no significant effect of rate of GA_3 on DM yields in this study, increased yields in response to higher rates of GA_3 application have been reported elsewhere (Percival 1980). The response to GA_3 at day 29 in this study was higher than that observed by Matthew *et al.* (2009) who applied

Table 2 Waikato dairy farm system model analysis: Effect of increasing period of GA_3 effect (one month for treatments 1–3, and 5 months for treatments 4–7) and extent of reduction in pasture N concentration (from 3.9%N in Base to 3.6%N in 1 and 4; to 3.4%N in 2 and 5; and 3.2%N in 3, 6 and 7, respectively) on pasture intake, excreta-N and N leaching estimated using a urine sub-model from OVERSEER®. In treatment 7 it was assumed that N fertiliser applications in April and August (30 kg N/ha in each) were omitted and the same production was achieved from use of GA_3.

		Treatment number						
	Base	1	2	3	4	5	6	7
Pasture DM intake (kg/ha/yr)	11065	11065	11065	11065	11065	11065	11065	11065
Pasture N intake (kg/ha/yr)	432	429	427	425	418	408	399	399
Total N excreted (kg/ha/yr)	353	350 (-0.8%)	348 (-1.3%)	346 (-1.8%)	339 (-3.9%)	329 (-6.5%)	321 (-9.5%)	321 (-9.5%)
Urine-N excreted (kg/ha/yr)	269	266 (-1.2%)	264 (-1.9%)	262 (-2.7%)	253 (-5.8%)	243 (-9.6%)	233 (-13.3%)	233 (-13.3%)
Urine-N excreted in April-August (kg N/ha)	80	78 (-2.7%)	76 (-4.5%)	75 (-6.1%)	69 (-13.7%)	62 (-22.3%)	55 (-30.5%)	55 (-30.5%)
N leaching from urine (kg N/ha/yr using OVERSEER®)	28	27	27 (-4%)	27 (-5%)	26 (-7%)	25 (-11%)	24 (-14%)	20 (-29%)

GA_3 at 0.3–0.4 times the GA_3 rates used in this study. When GA_3 was applied with N at 20 or 30 g GA_3/ha, the DM response increased by an average of 32% compared to urea alone. These findings are consistent with other studies reporting additive effects of application of GA_3 with N fertilisers (e.g. Matthew *et al*. 2009; Percival 1980). However, the magnitude of additive response in our studies conducted in spring was greater than that reported for autumn in other studies (Van Rossum *et al.* 2013; Zaman *et al.* 2014) which may be due to different growing conditions, i.e. warmer temperatures, improving the signalling impact of exogenous application of GA_3 resulting in greater cell elongation and cell division, combined with longer photoperiods encouraging greater photosynthesis in plants. Application of N together with GA_3 also did not prolong the pasture response period. This may be due to internal signalling mechanisms slowing down growth rate after an enhanced growth period to recuperate and maintain natural vigour. Parsons *et al*. (2013) have termed this mechanism as plants being "conservative" with resource use in order to maximise their long-term fitness.

Application of PGRs may adversely affect root biomass in pasture plants. However, shorter term observations (29–97 days after the treatment application) with a single application of GA_3 in our study showed no clear adverse effects on root biomass. These findings are consistent with those reported by Parsons *et al*. (2013) who observed no significant change in the root biomass of ryegrass plants when they were treated with GA_3 or GA_3 plus N. This aspect needs to be examined further for longer term or more frequent use of GA_3 in pastures.

GA_3 application significantly ($P<0.05$) decreased the total N concentration in pasture by about 0.5%N without N fertiliser or 0.3%N with N fertiliser at day 29 after treatment application (Figure 2). A related autumn study showed a decrease by up to 0.7%N (Zaman *et al*. 2014). Thus GA_3 application is a possible tool to reduce the concentration of N in herbage and thereby increase N efficiency for pasture production. In all cases with GA_3 application, the pasture N concentrations remained above the levels required for dairy cows for maximum milk production of 2.5–2.7% N (van Soest 1982; Luo & Kelliher 2010). Similarly concentration of K and P in the herbage remained satisfactory for animal feed quality (Roberts & Morton 2004) and were unaffected by GA_3 application. Pasture growth boosted by urea application generally increases pasture %N, particularly in the first 20 days after application (Figure 2). The greater the surplus intake of N in an animal's diet the greater is the amount of N in the urine fraction of the animal excreta (Kabreab *et al.* 2001; Luo & Kelliher 2010) which potentially increases nitrate-N leaching and gaseous loss of nitrous oxide (a greenhouse gas). Results from the Waikato dairy case farm analysis illustrated the potential benefits of GA_3 use on reducing N excretion in urine with associated decreases in N leaching from urine. Use of the Overseer® model showed that the largest calculated reduction in annual N leaching from urine was achieved when it was assumed that two N fertiliser applications were replaced by applications of GA_3 (Table 2).

Conclusions

This field study showed that a single application of GA_3 in early-spring on pastures is effective in boosting short term pasture production. In contrast, CCPU was ineffective in promoting plant growth at the application rates used here and none of the CCPU treatments in this trial showed any effect on DM yield. The pasture responses from GA_3 application were of a similar magnitude to those observed in the associated autumn trial (Zaman *et al*. 2014). There was no effect of differential GA_3 application rates on DM yields, because maximum yield was achieved using 20 g/ha GA_3. The pasture response from GA_3 was of a similar magnitude to that from urea application, suggesting that GA_3 may be a substitute option for boosting short-term pasture growth in autumn and/or early-spring.

GA_3 reduced pasture N concentration, which has potential benefits for decreasing N losses to the environment. Preliminary modelling indicated reduced N excretion by animals consuming GA_3 treated pasture and a decrease in urine-N leaching of up to 29% for a case study of a Waikato dairy farm.

ACKNOWLEDGEMENTS

The authors would like to thank Ballance Agri-Nutrients Limited for funding this research as part of the PGP programme. We thank John Waller for statistical analysis of data and Mike Sprosen for technical assistance with the trial work.

REFERENCES

Anderson, K.A. 1996. Micro-digestion and ICP-AES analysis for the determination of macro and micro elements in plant tissue. *Atomic Spectroscopy* Jan/Feb p 30.

Davies, P.J. 1995. Plant hormones, biosynthesis, signal transductions, action. Kluwer Academic Publishers, Dorchet, The Netherlands, Norwell, MA, USA.

Edmeades, D.C.; McBride, R.M. 2012. Evaluating the agronomic effectiveness of fertiliser products. *Proceedings of the New Zealand Grassland Association 74*: 217-224.

Kebreab, E.; France, J.; Beever, D.E.; Catillo, A.R. 2001. Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutrient Cycling in Agroecosystems 60*: 275-285.

Ledgard, S.; Webby, R.; Hawke, M. 2003. Improved estimation of N excretion by grazing animals to improve N_2O emission inventories. Report to MAF. AgResearch Ruakura. 27p.

Luo, J.; Kelliher, F. 2010. Partitioning of animal excreta N into urine and dung and developing the N_2O inventory. Report to Ministry of Agriculture and Forestry (MAF Pol 0910-11528, 09-03).

Matthew, C.; Hofmann, W.A.; Osborne, M.A. 2009. Pasture response to gibberellins: A review and recommendations. *New Zealand Journal of Agricultural Research 52*: 213- 225.

Parsons, A.J.; Rasmussen, S.; Liu, Q.; Xue, H.; Ball, C.; Shaw, C, 2013. Plant growth – resources or strategy limited: insights from responses to gibberellin. *Grass and Forage Science*, doi: 10.1111/gfs.12035

Percival, N. 1980. Cool-season growth response of kikuyu grass and ryegrass to gibberellic acid. *New Zealand Journal of Agricultural Research 23*: 97-102.

Roberts, A.H.C.; Morton, J.D. 2004. Fertiliser use on New Zealand dairy farms. Dairy Research Corporation, AgResearch and Fert Research, pp 40.

Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant, 2nd Edition, Ithaca, NY, USA, Cornell University Press.

Van Rossum, M.H.; Bryant, R.H.; Edwards, G.R. 2013. Response of simple grass-white clover and multi-species pastures to gibberellic acid or nitrogen fertiliser in autumn. *Proceedings of the New Zealand Grassland Association 74*: 183-187.

Zaman, M.; Ghani, A.; Kurepin L.V.; Pharis, R.P.; Khan, S.; Smith, T.J. 2014. Improving ryegrass-clover pasture dry matter yield and urea efficiency with gibberellic acid. *Journal of Food Science and Agriculture* DOI 10.1002/jsfa.6589.