

ASSESSMENT OF THE APPLICATION OF GIBBERELLINS TO INCREASE PRODUCTIVITY AND REDUCE NITROUS OXIDE EMISSIONS IN GRAZED GRASSLAND

David Whitehead ^{a,*} and Grant R. Edwards^b

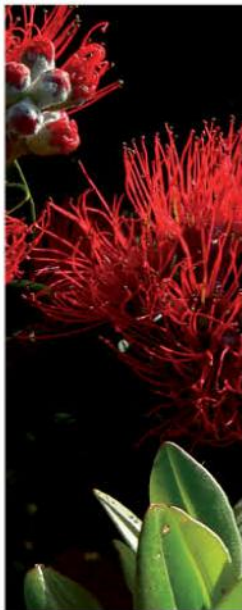
^a Landcare Research, PO Box 69040, Lincoln 7640, New Zealand

^b Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln 7647, New Zealand

*Corresponding author: Tel.: +64 3 321 9862, fax: +64 3 321 9998

E-mail address: whiteheadd@landcareresearch.co.nz (David Whitehead)

Co-author E-mail address: grant.edwards@lincoln.ac.nz (Grant R. Edwards)



Assessment of the application of gibberellins to increase productivity and reduce nitrous oxide emissions in grazed grassland*

David Whitehead^{a, *}, Grant R. Edwards^b

^a Landcare Research, PO Box 69040, Lincoln 7640, New Zealand

^b Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln 7647, New Zealand

ABSTRACT

Emissions of nitrous oxide from grassland systems are attributable largely to the use of nitrogen fertilisers and the excreta deposited by grazing animals. There is increasing interest in using gibberellins as a naturally-occurring growth promotant of herbage that could be used to reduce the use of nitrogen fertilisers while leading to similar or greater increases in dry matter. This may provide practical opportunities to reduce nitrogen intake by ruminants and to extend the seasonality of herbage growth in spring and autumn while reducing nitrogen losses resulting in lower rates of nitrogen excretion by grazing animals and reduced nitrous oxide emissions. Our findings from a review of previous studies confirm that gibberellins promote dry matter production, especially when applied in early spring or late summer/early autumn. When gibberellins are applied alone without nitrogen fertiliser, the nitrogen concentration of herbage is reduced and the impacts on forage quality are small and often not significantly different from those for untreated controls. We calculated the consequences of enhanced herbage production on nitrogen excreta returned to the soil as urine by a grazing dairy cow and estimated that one application of gibberellins will result in a relative reduction in nitrous oxide emission per urination event of 18% when compared with emissions from using nitrogen fertiliser. We used the OVERSEER[®] model and nitrous oxide emissions factors to estimate the impacts of changing herbage dry matter production, foliage nitrogen concentration and timing of one application of gibberellins on annual nitrous oxide emissions for a dairy farm. For one application of gibberellins in late summer and early spring, we estimate reductions in nitrous oxide emissions of 1.6% and 1.3%, respectively, relative to the response for an untreated control. Incorporating the effects of reduced use of nitrogen fertiliser by substituting one split application of fertiliser in late summer or autumn with gibberellins, we estimate reductions on nitrous oxide emissions of between 5 and 6% relative to the response for the untreated control. We conclude that the use of gibberellins with reduced addition of nitrogen fertiliser has the potential to reduce nitrous emissions from grazed grassland. However, acceptance of widespread use of gibberellins will be dependent on cost benefit analysis for farmers.

Keywords:

Forage quality, Gibberellins, Grassland production, Grazed grassland, Greenhouse gas mitigation, Nitrous oxide

*This was prepared initially under contract to the New Zealand Agricultural Greenhouse Gas Research Centre as Report LC2024, Landcare Research New Zealand Limited, December 2014

Contents

1.	Introduction	4
2.	Sources of nitrous oxide from grazed grassland	4
3.	Mitigation of nitrous oxide emissions.....	5
4.	Potential for use of plant growth promotants.....	5
5.	Physiological effects of gibberellins	6
6.	Effects of gibberellins on grassland dry matter production and seasonality.....	7
6.1	Dry matter production.....	7
6.2	Seasonality of production	8
6.3	Botanical composition of swards.....	8
7.	Effects of gibberellins on herbage nitrogen concentration and forage quality.....	9
8.	Estimation of the effects of gibberellins on nitrous oxide emissions	11
8.1	Calculations from nitrogen inputs and losses.....	12
8.2	Simulations using the OVERSEER® model.....	13
9.	Discussion and insights	17
	Acknowledgements	18
	References.....	19
	Appendix 1. Dairy farm parameters.....	23

1. Introduction

Despite its low concentration of 319 ± 12 ppbv in the atmosphere, nitrous oxide is the fourth most potent greenhouse gas because its high global warming potential associated with its long lifetime in the atmosphere of 120 years (Ramaswamy et al., 2001) is 298 times that for carbon dioxide (Forster et al., 2007). Globally, more than 40% of the emissions of nitrous oxide are attributable to agricultural systems managed for grazing animals (Denman et al., 2007) and emissions have continued to increase during the last two decades. Nitrous oxide is derived from microbiological processes in the soil acting on nitrogen that is applied as fertiliser, mineralised from stored organic sources or biologically fixed from atmospheric nitrogen. Grazing livestock are responsible for additional emissions from dung and urine deposited by the animals directly or from controlled application of stored waste.

New Zealand's greenhouse gas inventory is unique among OECD (Organisation for Economic Co-operation and Development) countries in that agricultural emissions of methane from enteric fermentation in the rumen of grazing animals (37.0% of gross emissions) and nitrous oxide (14.2% of gross emissions) are high relative to the emissions from carbon dioxide (Ministry for the Environment, 2013). This is attributable to the large surface area of land (42%) devoted to grazed grasslands with agricultural products being the dominant contributor to the economy (NZDairy and LIC, 2012).

Increasing intensification of livestock farming on grasslands is facing a growing challenge. Most notable is the recent widespread increases in the scale and intensity of the conversion of dryland to irrigated grassland to support dairy farming. Economic pressures to increase the supply of forage for animals and associated agricultural products are colliding with growing concerns of increased requirements for resources (e.g., nitrogen and water) and the impact of high resource use on the environment. In New Zealand, particular concerns are the increases in nitrous oxide emissions and nitrate leaching. There are similar concerns in northern Europe, even though nitrous oxide emissions have reduced in some countries with decreased intensity of grassland production with the implementation of agri-environmental policies (European Environment Agency, 2014).

2. Sources of nitrous oxide from grazed grassland

Countries that are signatories to the United Nations Framework Convention on Climate Change (UNFCCC) are required to provide annual inventories of greenhouse gas emissions and removals. Data for New Zealand show that nitrous oxide emissions increased by 29% from 25.4 to 32.8 Gg N year⁻¹ between 1990 and 2011 (Ministry for the Environment, 2013). Nitrous oxide emissions from grazed grassland, notably dairy farms, occur because the inputs of nitrogen fertiliser to maintain high herbage and animal productivity exceed the ability of soils to retain the nitrogen. Surplus nitrogen is lost through nitrate leaching and emissions of gaseous nitrogen in the forms of ammonia, nitric oxide, nitrous oxide and nitrogen gas (Luo et al., 2010).

Emissions of nitrous oxide are regulated by interactions between soil physical and chemical properties, soil microbiological populations, climate, and animal management practices. Emissions are enhanced by the deposition or controlled application of animal excreta to the soil especially during anaerobic conditions resulting from wet soil conditions and soil compaction from animal treading (Saggar, 2004, 2009; de Klein and Ledgard, 2005; van der Weerden et al., 2014). The potential for emissions is thus higher in winter when soils are wet and more vulnerable to compaction.

For inventory purposes, it is necessary to calculate nitrous oxide emissions from each source independently. The UNFCCC (United Nations Framework Convention on Climate Change) approach is to apply relative direct emission factors (EFs) to each source that expresses the proportion of the quantity of nitrogen applied to soils that is emitted to the atmosphere as nitrous oxide. Emission factor EF₃ is used to estimate nitrous oxide emissions from urine and dung deposited during grazing. Values for EF₃ are obtained from measurements of nitrous oxide emissions when known amounts of urine and dung are applied to soils (van der Weerden et al., 2014). Emission factor EF₁ refers to nitrous oxide emissions from

the addition of nitrogen fertiliser to grassland. In a recent statistical analysis of measurements of nitrous oxide emissions from 185 field sites in New Zealand, Kelliher et al. (2014) concluded that the appropriate (mean \pm standard error) values of EF_3 for dairy cattle urine and dung, and sheep urine and dung are $1.16 \pm 0.19\%$, $0.23 \pm 0.05\%$, $0.55 \pm 0.19\%$ and $0.08 \pm 0.02\%$, respectively. The higher values of EF_3 for both dairy cattle and sheep urine compared with those for dung are attributable to the higher concentrations of mineral nitrogen that are deposited in a small area. Analysis of New Zealand's available data for the emission factor from the addition of nitrogen fertiliser as urea, EF_1 , by Kelliher et al. (2014) showed that the value is $0.48 \pm 0.13\%$. We have used this value in anticipation that it will replace the current value of 1% used in the national inventory (Ministry for the Environment, 2013).

3. Mitigation of nitrous oxide emissions

Various approaches have been identified to mitigate greenhouse gas emissions associated with grazed grassland systems (de Klein and Ledgard, 2005). These include soil management, animal management using feed pads or housing systems, controlled application of effluent, reductions in nitrogen fertiliser, lowering the amount of nitrogen excreted by animals, use of feed with low nitrogen content, selecting animals with high nitrogen use efficiency, and the use of chemical inhibitors that block the conversion of urea to ammonium and ammonium nitrate in soils (Luo et al., 2010). Rates of nitrous oxide production are dependent on complex interactions between soil properties, soil microbiology, climate and management practices (Saggar et al., 2009). Rather than the supply of nitrogen from urine and dung deposits and nitrogen fertiliser, in New Zealand grasslands nitrous oxide emissions are regulated primarily the rates of denitrification and its dependence on soil water content (Müller and Sherlock, 2004; van der Weerden et al., 2014).

To reduce nitrous oxide emissions from grassland, in terms of animal-based approaches, there is a need to focus on the root cause of the problem, which is primarily the excess amount of nitrogen excreted, particularly in urine patches. Ruminants have an optimum nitrogen (N) concentration in the diet of around 2.5%, equivalent to a crude protein (CP) concentration of 15.6% (i.e. $6.25 \times N\%$) (van Soest, 1982). However, Parsons et al. (2013) report that to maximise the forage supply to animals per unit land area requires a nitrogen concentration in leaves of around 4.5% for most cool temperate grassland species (e.g., perennial ryegrass, *Lolium perenne* L.) to reach maximum photosynthetic capacity for the sward (Woledge and Pearse, 1985). This leads, in turn, to reliance on the use of nitrogen-based fertilisers to increase pasture growth. These contrasting requirements – of high nitrogen in herbage to maximise pasture growth and low to moderate herbage nitrogen concentration for animal production – lead to excessive nitrogen intake per animal and large amounts of nitrogen excreted in urine. This leads to high nitrogen loading in urine patches (from 700 to 1 000 kg N ha⁻¹ for dairy cows; Haynes and Williams, 1993) of labile mineral nitrogen that is prone to loss as nitrous oxide and nitrate leaching. Thus, a logical pathway in grazed grassland systems to controlling nitrogen surplus in the animal is to manipulate the nitrogen content of the herbage consumed. In grassland production systems, where feed supplement is often minimal, the amount of excess nitrogen can be regulated by the amount and nitrogen content of the herbage consumed.

4. Potential for use of plant growth promotants

To address this issue, there is increased interest in naturally-occurring growth promotants (or biostimulators) of herbage that could be used to reduce the use of nitrogen fertilisers (e.g., replace the use of urea), while potentially leading to similar or greater increases in dry matter (DM) yield (Kurepin et al., 2014). Further, if the increased dry matter yield could be delivered without increases in the nitrogen content of herbage per unit area that is usually associated with nitrogen fertiliser, there may be practical opportunities to reduce nitrogen intake by ruminants to better align the amounts with the optimum values for animal production. Gibberellins, plant shoot growth-promoting phytohormones, offer potential in this role. Through integration of plant performance with the optimal climatic variables and

grassland management, plant hormones have the potential to improve herbage production, within the context of environmental constraints associated with the nitrogen cycle. The effectiveness of applications of gibberellins on promoting herbage production is likely to be more pronounced in early spring and late autumn when the activation of endogenous levels regulated by phytochrome-sensed changes in photoperiod (Kurepin et al., 2011) is more limited by lower temperatures (Kurepin et al., 2013).

Matthew et al. (2009) undertook a comprehensive review of grassland response to gibberellins using findings from 72 responses across a range of grass, legume and herb species. Adding gibberellins may provide an alternative to increase pasture production when growth is limited in spring and autumn. The authors concluded that application of gibberellins at low concentrations can produce an economically cost-effective increase in herbage production in autumn and spring and allow the seasonality of production to be manipulated. Much of the earlier work has been undertaken with applications of gibberellins in spring and data are lacking for the effects of adding gibberellins in autumn. Further, the early work reviewed by Matthew et al. (2009) showed marked differences in the response of plant species to application of gibberellins, resulting in changes in the botanical composition of mixed swards.

Our objectives in writing this review are to incorporate the findings from more recent studies with those of Matthew et al. (2009) to address the potential for the use of gibberellins to increase herbage production. We then extend the scope to include the impacts on the nitrogen cycle for grazed grasslands with particular focus on reducing nitrous oxide emissions. We undertake this by reviewing the literature then use the findings in a farm grazing model to simulate the impacts on annual herbage production and the nitrogen cycle using a case study of a representative dairy farm in New Zealand.

5. Physiological effects of gibberellins

The discovery of gibberellins as a regulator of plant growth is attributed to Japanese researchers working with extracts from the fungus complex *Gibberella fujikuroi* (Brian et al., 1954). They found that application of the extracts to plants produced symptoms of excessive elongation. Subsequently, the search for ways to increase global food production starting in the 1950s led to isolation of 50 known gibberellins, but gibberellic acid GA₃ is most commonly available and used commercially. In addition to manipulating shoot elongation, gibberellins are now known to affect a wide range of plant growth and development and phenological events (Davies, 1995). Gibberellins are involved in mineral regulation (Kiba et al., 2011) and they influence resource allocation within plants between roots, shoot and reproductive material (Brenner, 1987), and affect flowering (Funnell et al., 1992) and fruit quality (Drake et al., 1990) in horticultural crops. The physiological effects of gibberellins are known to result from their activation of signalling processes at the molecular level. These signals activate dormant enzyme systems and trigger the degradation of proteins that restrain shoot elongation (Schwechheimer, 2008).

Early studies used applications of gibberellins in high concentrations (up to 700 g active ingredient ha⁻¹) reported by Matthew et al. (2009). With high manufacturing costs, early studies concluded that the use of gibberellins for increasing herbage production in grasslands was not economically viable (Percival 1980). The analysis by Matthew et al. (2009) showed that the response per unit mass of gibberellins applied decreased with increasing rates of application. With much reduced costs, increased availability, and realisation of the effectiveness of gibberellins applied at low rates of application (5–10 g active ingredient ha⁻¹ reported by Matthew et al. (2009)), there is increasing interest in their commercial use to improve grassland management. Critical to the success will be the contribution of gibberellins to manipulating plants to overcome 'strategy' limitations for resource use (Ball et al., 2012; Parsons et al., 2013) and optimising farm management systems (Matthew et al., 2009). Specific focus is on the ability of gibberellins to extend the seasonality of herbage growth in spring and autumn by the mobilisation of resources resulting in leaf and stem elongation while reducing nitrogen inputs and losses.

Earlier studies showed some side effects of application of gibberellins including late-yield depression likely attributable to plant reserve utilisation in winter (Mitchell, 1956), increased shoot to root ratio, accentuated with reduction in root growth when nitrogen fertiliser is added (Champ  roux, 1962), decreased tillering and number of seed heads, and increased apical dominance. The stimulation of flowering acted in the same way as increased day length or exposure to cold temperatures. Yellowing from chlorophyll reduction was also observed but this is overcome by adding nitrogen. There are other possible effects of addition of gibberellins related to canopy structure with more apical dominance and changes to interception of irradiance, but these are likely to be minor.

6. Effects of gibberellins on grassland dry matter production and seasonality

6.1 Dry matter production

The response of herbage dry matter production to application of gibberellins has important implications for nitrous oxide emissions because herbage supply is a key component of determining overall nitrogen intake and the number of animals that can be supported per unit area of grassland. The extent to which gibberellins may substitute for, or work in conjunction with co-application, of nitrogen fertiliser, and the seasonality of these effects relative to typical patterns of plant growth and soil water status are critical for the potential for nitrous oxide emissions and nitrate leaching. The extensive review of the effects of application of gibberellins on dry matter production by Matthew et al. (2009) from 72 observations with pots and field plots made between 1957 and 2009 in Europe, USA, Australia and New Zealand across a range of grass, legume and herbaceous species found that the highest response was an increase in dry matter production of 2 000 kg DM ha⁻¹. However, the average increase in dry matter production relative to that for untreated controls without nitrogen fertiliser applied was 500 kg DM ha⁻¹. It is important to note that such increases in annual dry matter yield may be difficult to detect with certainty because of spatial variability and limitations of techniques for precise measurements. In an additional experiment with ryegrass clover swards in small plots and careful measurements for dry matter production, Matthew et al. (2009) showed that application of gibberellins in spring increased the height of the herbage relative to untreated controls without nitrogen fertiliser applied but did not increase dry matter production when the plots were grazed after one month. The second grazing event after a further month showed that there was no residual effect of gibberellins on dry matter production. The plots where gibberellins were applied in the absence of nitrogen fertiliser showed lower yield and tiller density, suggesting a second-cut yield depression.

More recent studies conducted across New Zealand with a perennial ryegrass and white clover (*Trifolium repens* L.) mixed grassland have noted yield increases of 212– 1199 kg DM ha⁻¹ in response to application of gibberellins at 20 g active ingredient ha⁻¹ relative to that for untreated controls without nitrogen fertiliser applied, with a mean response of 427 kg DM ha⁻¹ (Van Rossum et al., 2013; van Rossum, 2013; Zaman et al., 2014a). In the same set of trials, the response to application of 40 kg N ha⁻¹ as urea fertiliser relative to that for unfertilised pasture not treated with gibberellins ranged from 40 to 767 kg DM ha⁻¹ relative to that for untreated controls, with a mean response of 384 kg DM ha⁻¹. The commercial company NuFarm (Edmeades, 2009) undertook 35 replicated field trials across New Zealand using the manufactured gibberellin ProGibb SG. Gibberellins were applied at the rate recommended by the supplier within 0–5 days following a grazing event. This resulted in an increase in average production relative to that for untreated controls without nitrogen fertiliser applied of 36% with a range of 12% to 63%, provided the pasture is grazed within 40–50 days following application. Where gibberellins have been applied simultaneously with nitrogen fertiliser, the effects have been shown to be additive (van Rossum et al., 2013; van Rossum, 2013; Zaman et al., 2014a, b).

6.2 Seasonality of production

Early studies (Scurfield and Biddiscombe, 1959; Biddiscombe et al., 1962) demonstrated that the effect of application of gibberellins was evident early in the growing season (e.g., spring), with a reduced effect in promoting growth in summer and early autumn. These findings are confirmed by Matthew et al. (2009) and Edmeades (2009), particularly for cool temperate perennial ryegrass and white clover grassland. Parsons et al. (2013) undertook a more detailed analysis of the effect of gibberellins on the growth of perennial ryegrass as affected by plant developmental state and the interaction with growing conditions. Plants taken from the field in winter and maintained under controlled conditions to sustain their winter developmental state (e.g., short days) showed a significant capacity to respond to application of gibberellins relative to that for the untreated controls. In contrast, plants taken from the field in summer and maintained under controlled conditions to sustain their summer developmental state (e.g., long days) showed very low capacity to respond to gibberellins. Because responses to gibberellins were shown to depend strongly on the time of the year when plants were treated, rather than conditions at the time of the measurement period, this indicates gibberellins may be interacting with a fixed seasonal growth strategy of plants. Current conditions at a given time will, however, be important in determining the rate of growth of plants in response to gibberellins. For example, plant growth rate will be restricted in field conditions when temperatures are low. The finding that the potential to respond is greater in plants at the end of winter is consistent with the most marked responses to gibberellins occurring as temperatures increase in spring (Parsons et al., 2013).

6.3 Botanical composition of swards

In addition to effects of gibberellins on dry matter production, it is important to consider the implications of changes in species composition on forage quality resulting from differing responses to gibberellins among plant species. This may lead to changes in composition of diet consumed (e.g., amount of nitrogen) by animals and, thereby, an effect on nitrous oxide emissions through altered nitrogen excretion in urine and dung (Dijkstra et al., 2013). Measurements of nitrous oxide released from different forage mixes following grazing show that emissions from pure legume crops are higher than those from non-legume and legume/grass mixes (Rochette and Janzen, 2005). The enhanced emissions from legume swards are due not only to higher nitrogen returns from the animal excreta, but also to more rapid decomposition of plant litter that is high in nitrogen (Galbally et al., 2010). Further, nitrous oxide emissions may depend on botanical composition, reflecting species-specific effects related to differences in shoot and root growth rates (Abalos et al., 2014). Of particular relevance are the impacts of gibberellins on the balance of grasses, legumes and herbaceous species in the sward.

There are few studies that have compared the response of different species to application of gibberellins within the same trial. Early qualitative work by Wittwer and Bukovac (1957) with C3 grass species showed that Kentucky bluegrass (*Poa pratensis* L.) was more responsive to gibberellins than perennial ryegrass when applied at the high application rate of 75 g active ingredient ha⁻¹. This finding was supported by the work of Finn and Nielsen (1959). Another indication of the lower response of perennial ryegrass is that when gibberellins were applied to an old pasture, the percentage of perennial ryegrass decreased from 63% to 53% in favour of *Agrostis* spp. and Yorkshire fog (*Holcus lanatus* L.) (McGrath and Murphy, 1976). Similar responses to gibberellins have also been observed in C4 grass species including kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov), Bermuda grass (*Cynodon dactylon* (L.) Pers.), and paspalum (*Paspalum dilatatum* Poir.) (Lester and Carter, 1970; Whitney, 1976). Differences in the response of legumes have been observed in several studies. Finn and Nielsen (1959) showed that three legume species – lucerne (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.) and white clover – were more responsive than the three grasses, particularly at the high rates of gibberellin application of 295 and 590 g active ingredient ha⁻¹. Other studies have noted that gibberellin application may inhibit nodulation (Thurber et al., 1958) although this is not always the case (Fletcher et al., 1958). The

herbaceous plants chicory (*Chicorium intibus* L.) and plantain (*Plantago lanceolata* L.) showed a positive response (Wittner and Bukovac, 1957; Dijkstra et al., 1990), when gibberellins were applied in spring when plants were approaching the reproductive stage.

It is important to note that the response of pure stands or plants growing in pots may not always be a good indicator of responses to mixtures when the species are subject to interspecific competition in the field. Biddiscombe et al. (1962) reported a reduction in the proportion of subterranean clover (*Trifolium subterranean* L.) in *Phalaris*-dominated grassland treated with gibberellins compared with untreated plots. Van Rossum (2013) showed that a single application of gibberellins applied in both spring and autumn resulted in an increase in the mass of both perennial ryegrass and white clover in the pasture. However, when expressed on a relative basis, the percentage of white clover was higher in autumn (33% instead of 29%) and spring (17% instead of 12%) in the plots treated with gibberellins. A further comprehensive study between a simple grassland with the two species (ryegrass or tall fescue and white clover) and multi-species mixtures (ryegrass or tall fescue with white clover, chicory and plantain) showed that the effect of gibberellins was dependent on the sensitivity of the base grass species to the treatment relative to that of either herbs or legumes in the mixture (van Rossum et al., 2013). Application of gibberellins increased the percentage of white clover in all treatments except the tall fescue – white clover pasture, where a strong response of tall fescue to gibberellins resulted in a reduction in the proportion of white clover in the mix. The portion of herbaceous species in the multi-species mixture was increased by addition of nitrogen (31.8% vs 37.9%) but not affected by gibberellins (35.1% vs 35.6%). These findings lead to the conclusion that, when gibberellins are applied to mixed pastures, this is likely to result in increased dry matter production for most species. However, because of enhanced dry matter production by legumes, this may result in an increase in the proportion of the legumes, e.g., clover. This contrasts with the addition of nitrogen fertiliser, which results in increased dry matter production but enhanced growth of grasses, resulting in a reduction in the proportion of legumes in the sward.

7. Effects of gibberellins on herbage nitrogen concentration and forage quality

The chemical composition of forage is critical for nitrous oxide emissions through the effects on the quantity of nitrogen excreted and the number of urine patches per unit area. There are three key considerations of effects of gibberellins on forage composition. The first is the effect of gibberellins on herbage nitrogen concentration. As reported above, excreta, particularly urine, are the primary source of nitrogen for nitrate leaching in grazed grassland systems. The main factor affecting the amount of nitrogen excreted is the quantity of nitrogen consumed by animals (Higgs et al., 2013). Further, it has been highlighted that the nitrogen or crude protein (CP) content of herbage is often in excess of requirements for animal production. Reducing the nitrogen content of the feed with the same dry matter intake has the potential to reduce nitrogen intake and the proportion of nitrogen retained in the products from animals (mainly meat and milk). The second consideration is the effect of gibberellins on the water soluble carbohydrate (WSC) concentration of the herbage. Feeding fresh forages with a high water sugar concentration has been proposed as a way to address imbalances, or asynchrony, in energy and protein availability for microbial protein synthesis by animals (Beever et al., 1986). This in turn decreases the concentration of ammonia in the rumen (Miller et al., 2001) and the subsequent nitrogen loss in excreta. Improved WSC concentrations in the diet have rarely resulted in improvements in animal performance. However, more consistent prospects of reducing the proportion of dietary nitrogen excreted in urine have been shown, particularly where the WSC:CP ratio can be increased beyond a ratio of 0.7, equivalent to a WSC:N ratio of 0.1 (Edwards et al., 2007). The third consideration is the effect of gibberellins on the metabolisable energy content per unit dry matter (ME) of the herbage. Increasing dietary ME concentration relative to nitrogen intake may increase nitrogen capture in the rumen and reduce urinary nitrogen output (Dijkstra et al., 2013). Further, an improvement in dietary ME may enhance the potential productivity of individual animals. This may potentially result in lower nitrogen excretion per animal. If increased productivity per animal is associated with fewer animals, there may also be lower nitrogen excretion per unit area, particularly when the need for fewer replacement stock is considered (Dijkstra et al., 2013). The final consideration is the effect of gibberellins on the mineral

composition of the herbage. Increasing dietary mineral content is linked to increased urine volume (Dijkstra et al., 2013), which may reduce nitrous oxide emissions for the same amount of urine nitrogen (Groenigen et al., 2005).

In a mixed sward, overall herbage quality and nitrogen content will depend on the relative abundance and response to gibberellins for each species. Early work with individual species showed that nitrogen concentrations in Kentucky bluegrass, perennial ryegrass, white clover, lucerne and birdsfoot trefoil were reduced following the application of gibberellins (Finn and Nielsen, 1959; Brown et al., 1963), although Morgan and Mees (1958) detected no effect. In more recent work with individual plants growing in controlled environments, Parsons et al. (2013) undertook detailed analyses of forage composition of harvested herbage (above a height of 60 mm), stubble (herbage below a height of 60 mm) and roots for perennial ryegrass growing in pots in controlled conditions following applications of gibberellins and nitrogen singly and in combination. No significant interactions on forage composition were detected between the treatments where gibberellins and nitrogen were applied. Higher nitrogen resource supply was associated with higher concentrations of low molecular weight (e.g., sucrose, glucose, fructose) and high molecular weight (e.g., fructans) water soluble carbohydrates in all tissues, and increased the nitrogen concentration of all tissues, leading to a major reduction in the WSC:CP ratio in all tissues. In contrast, application of gibberellins resulted in little change in either high- or low-molecular-weight sugars in herbage harvested above a height of 60 mm, and little change in the nitrogen content of the herbage relative to plants that were not treated with gibberellins. As a consequence, there was no significant change in the WSC:CP ratio of the harvested herbage as a result of the application of gibberellins.

In a series of field trials in Canterbury (South Island) and Waikato (North Island, New Zealand), Zaman et al. (2014a) investigated the effects on dry matter yield, herbage nitrogen concentration and forage composition for ryegrass and white clover swards of spraying gibberellins with and without nitrogen fertiliser applied as liquid urea. The herbage ME value was reduced by application of gibberellins relative to that of the control not fertilised with nitrogen. However, co-application of gibberellins with nitrogen restored the ME values to those for the control or nitrogen-treated plots. Herbage nitrogen concentration was reduced by 5.1 g N kg DM⁻¹ (0.5% N) by application of gibberellins alone relative to that for untreated control plots, while nitrogen application resulted in similar herbage nitrogen concentrations relative to those for the untreated control plots. Of note is that application of nitrogen and gibberellins together increased dry matter yield with the herbage nitrogen concentration lower than the value for the untreated control plots by 3 g N kg DM⁻¹. Herbage WSC values were unaffected by addition of nitrogen or gibberellins.

In related work on the application of gibberellins, Ghani et al. (2014) showed that total nitrogen content of grassland was decreased by about 0.5% with no application of nitrogen fertiliser and by 0.3% when nitrogen fertiliser was applied. Van Rossum et al. (2013) associated the effect of application of gibberellins on herbage nitrogen concentration with changes in the proportion of clover in the swards. In multi-species and tall fescue with clover swards where the proportion of clover increased only marginally in response to application of gibberellins, herbage nitrogen concentration was similar in plots treated with gibberellins and the untreated controls. However, herbage nitrogen concentration on plots treated with gibberellins was 6–7 g N kg DM⁻¹ (0.6 to 0.7% N) lower than the values in plots where nitrogen was added. In ryegrass–clover swards there was an increase in herbage nitrogen concentration on plots treated with gibberellins compared with untreated plots because the proportion of white clover increased from 1% to 10%. However, overall the nitrogen content was similar to that for the plots with added nitrogen fertiliser. Application of nitrogen and gibberellins resulted in an additive effect on dry matter, but the average nitrogen concentration of herbage was 2.2 g N kg DM⁻¹ (0.22%) lower in plots treated with gibberellins than in those treated with gibberellins and nitrogen. Application of gibberellins had little effect on ME or WSC of the harvested herbage relative to values for the untreated controls.

In studies of temperate grasslands, the demonstrated effects of application of gibberellins either alone or in combination with nitrogen fertiliser on herbage ME or WSC have been shown to be small (Matthew

et al., 2009; van Rossum et al., 2013; Zaman et al., 2014a). Few studies have measured neutral detergent fibre (NDF) values but van Rossum et al. (2013) showed that these were unaffected by application of gibberellins. Further, although CP was reduced by application of gibberellins, it was generally higher than critical values required for animal production. These data indicate that the effects of application of gibberellins on animal performance operating through changes in herbage nutritive value may be minimal. However, this does not negate the possibility of effects on animal performance that may result from changes to sward characteristics following application of gibberellins. For example, application of gibberellins typically results in taller pastures, and on occasion a lower tiller density, and these two factors can influence the feed intake by animals (Cosgrove and Edwards, 2007). There are no direct studies of the effect of application of gibberellins on foraging behaviour of livestock. However, Allen (2010) investigated milk production in autumn of late lactation dairy cows allocated the same quantity of herbage treated with either gibberellins or nitrogen fertiliser. Grassland quality as indicated by ME content was unaffected by treatment, and milk production was similar for the plots treated with gibberellins (1.45 kg MS cow⁻¹ day⁻¹) and nitrogen (1.42 kg MS cow⁻¹ day⁻¹). This confirms that when provided in equal quantities with nitrogen, it is not expected that application of gibberellins will affect livestock performance. These findings confirm earlier work by Percival (1980) who showed that reduced crude protein levels from application of gibberellins were usually offset by increased yield leading to increased protein yield per unit area. This resulted in increased live weight of sheep, suggesting there was no decrease in herbage nutritive value. From measurements using near-infrared spectroscopy, Matthew et al. (2009) found no decrease in forage quality following application of gibberellins. Any effect of gibberellins on livestock performance is more likely to be associated with the effect of application of gibberellins on the amount of herbage grown relative to that for untreated plots or plots with added nitrogen fertiliser.

There is little information on the mineral composition of herbage following application of gibberellins. Gibberellins had little impact on the concentration of minerals in perennial ryegrass, with the one exception being sodium (Na) uptake, which was increased by over 1.4 g Na kg DM⁻¹ compared with that for the control treatment (R. Bryant, unpublished data). The reasons are not clear though increased sodium in herbage treated with gibberellins could be associated with increased water demand from stimulated growth and increase in cell expansion and cell volume and changes in sugar transport, which are all regulated in some way by gibberellins.

8. Estimation of the effects of gibberellins on nitrous oxide emissions

If application of gibberellins reduces the nitrogen concentration in herbage, then there is the potential for gibberellins to reduce nitrogen intake by animals, so leading to reduced nitrogen concentration in urine, lower total nitrogen excretion and reduced nitrous oxide emissions. To test this, we used two approaches. Firstly, using calculations of nitrogen inputs and outputs for a dairy herd we estimate the impact of change in herbage nitrogen concentration that could result from the use of gibberellins, on nitrogen losses for an individual urination event. Secondly, we use the farm systems model OVERSEER® (Wheeler et al., 2003; Wheeler, 2014), which is used commercially to estimate nitrogen inputs and outputs for dairy herds at farm scales, to integrate the effects of application of gibberellins on seasonal and annual nitrous oxide emissions.

The OVERSEER® model partitions the excreted nitrogen in urine and dung (de Klein and Ledgard, 2005; Wheeler et al., 2013) after accounting for the amounts of nitrogen removed in animal products. The beta version of the model we used is not yet available commercially but allowed simulation of seasonal changes in herbage intake and nitrogen concentration to be incorporated into the calculations. To focus our calculations on the impacts of applications of gibberellins and nitrogen fertiliser, we based the scenarios in the model on a simplified farm system (application of 200 kg N ha⁻¹ year⁻¹) for a typical commercial dairy farm in the Waikato Region, New Zealand. With annual rainfall of 1 138 mm irrigation of the pasture is not required and the year was selected when there was no drought. We assumed that all effluent, solids and liquid were exported, that there was no imported supplementary feed on-pasture

or in-shed and a feed pad was not used. The annual dry matter production of herbage for the farm is 16.1 kg DM ha⁻¹ year⁻¹ and further details of the location, climate and farming system are shown in Appendix 1. The physiological effects of gibberellins on the urination frequency and volume of the animals is not known. So, for simplicity, we assumed that these are not affected by changes in species composition of the pasture or herbage quality that may result from application of gibberellins. We used the same input variables for both approaches. Based on the findings from our review of the literature, our assumption is that, when gibberellins or nitrogen are applied once in early spring (August) or late summer (February), both achieve a similar increase in dry matter production over a one month period compared with that for the control of 500 kg DM ha⁻¹ year⁻¹. However, we do allow for the total herbage nitrogen content in swards treated with gibberellins to be 0.4% (3.6% vs 4% N) lower than the value for swards treated with nitrogen. We further assume that a single application of gibberellins and nitrogen fertiliser have no ongoing effects on production or foliage nitrogen concentration in subsequent months. We combine the outputs from our calculations with the emissions factors reported by Kelliher et al. (2014) to estimate changes in nitrous oxide emissions. We include direct nitrous oxide emissions from urine, dung and the application of nitrogen fertiliser but, for simplicity, we ignore the indirect nitrous oxide emissions from these three sources as well as emissions associated with the manufacture of urea.

8.1 Calculations from nitrogen inputs and losses

We demonstrated the effects of application of gibberellins by example under dairy cow grazing, assuming that either gibberellins or nitrogen could be applied post-grazing in mid- autumn and that both achieve a similar increase in herbage dry matter yield of 500 kg DM ha⁻¹ over a one-month period compared with the production for the untreated control. For a late lactation dairy cow eating 15 kg DM day⁻¹, the reduction in total herbage nitrogen content of 0.4% for swards treated with gibberellins compared with those treated with nitrogen corresponds to a nitrogen intake of 540 and 600 g N cow⁻¹ day⁻¹, for the gibberellins and nitrogen treatments, respectively. Using the equation from Tas (2007), excretion in urine (g N cow⁻¹ day⁻¹) – 147.5 + 0.812 × nitrogen intake (g N day⁻¹), would give urinary nitrogen excretion of 291 and 340 g N cow⁻¹ day⁻¹ for the gibberellin and nitrogen treatments, respectively. These data indicate that the changes in nitrogen intake delivered by application of gibberellins have the potential to reduce nitrogen excretion in urine.

Assuming that urination frequency and volume is not altered by application of gibberellins compared with those for the treatment with nitrogen fertiliser and emission factors for dairy cattle urine of 1.16% would give relative reduction in nitrous oxide emission per urination event of 14% when using gibberellins compared with emissions using nitrogen fertiliser.

This analysis is based on several key assumptions. Firstly, we assume that the urination patterns (volume, frequency) and composition of the urine other than nitrogen concentration are similar. Changes in urine volume and composition (e.g., hippuric acid, purine derivatives) may affect nitrous oxide emissions (Dijkstra et al., 2013). However, differences in herbage and mineral composition that drive variation in urine characteristics, with the exception of increased sodium concentration with herbage treated with gibberellin, are relatively small (R. Bryant, unpublished data), indicating that any changes in urine characteristics with application of gibberellins would also be minimal. Our second assumption is that changes in botanical composition with application of gibberellins did not affect the emission factors. Changes in species composition, particularly an increase in the proportion of legumes, have been reported with application of gibberellins, but whether these are sufficient to alter emission factors, particularly when all are at low abundance, post-grazing is unknown.

The example outline above considers the effects of a single application of gibberellins compared with application of nitrogen fertiliser, with effects of gibberellins on herbage composition considered only within the regrowth period immediately following application. A recent analysis by van Rossum (2013) showed that the effect on forage composition was not sustained in subsequent regrowth periods. The nitrogen content of grassland plots with no additional treatment following the application of gibberellins following nitrogen application in the previous regrowth was similar to the

nitrogen content for the control plots. This suggests that repeated applications of gibberellins will be required to extend the period over which gibberellins affect nitrous oxide emissions. In this context, concerns have been raised over the effects of sequential applications of gibberellins on sward growth, including those associated with drawing down of mineral nitrogen in the soil (Parsons et al., 2013) and depletion of root reserves (Matthew et al., 2009). Further, there is evidence that successive applications of gibberellins in the absence of nitrogen will reduce tillering in ryegrass. However, there are few data investigating the effect of sequential applications of gibberellins on herbage dry matter production. A current study underway at Lincoln University is investigating the effects of multiple applications of gibberellins and nitrogen on herbage dry matter production and botanical composition by applying 2, 4, 6 or 10 applications of gibberellins (half in spring and half in autumn). Findings from the first year show that there is no negative effect on dry matter yield from as many as 10 applications of gibberellins when supplied simultaneously with nitrogen (R. Bryant, unpublished data). Further, data indicate that in situations where nitrogen fertiliser is applied after each grazing (approximately 10 times per year), nitrogen fertiliser could be replaced with gibberellins for two applications in spring and autumn without a significant reduction in total annual dry matter yield. On this basis the window of opportunity for using gibberellins to reduce nitrous oxide emissions could be extended to four grazing events per year without a significant decline in dry matter production.

8.2 Simulations using the OVERSEER® model

For the scenarios using OVERSEER® we selected six treatments and compared the outputs with a control treatment (Table 1). For the control treatment, nitrogen fertiliser was applied in four split dressings each of 50 kg N ha⁻¹ in early spring (August), spring (October), summer (December) and late summer (February). The treatments consisted of single applications of gibberellins either in late summer (February) or early spring (August) with or instead of applications of nitrogen. We also allowed for the effects of applications of gibberellins to result in no change or a reduction in herbage nitrogen concentration by 0.5%.

The annual nitrogen excreted by the cows for the control treatment (scenario 1) is 493 kg N ha⁻¹ year⁻¹ (Table 2). Highest excretion rates occur in spring and summer when herbage dry matter production for grazing is high. Consistent with the treatments we applied (Table 1), rates of nitrogen excretion are lower than the rate for the control treatment during the month when the application of gibberellins resulted in a decrease in herbage nitrogen concentration (scenarios 2 and 5 in late summer and 5 and 6 in early spring). There are no carry-over effects of the treatments into months following the month of application and the subsequent month.

Results from the model show that single applications of gibberellins in late summer (scenario 4) and early spring (scenario 7) with an additional increase in herbage dry matter production of 500 kg DM ha⁻¹ and no reduction in herbage nitrogen concentration resulted in annual increases in nitrogen intake compared with that for the control treatment (scenario 1) by 2.6% and 2.5%, respectively (Table 3). This in turn led to annual increases in the amounts of nitrogen deposited as urine and dung of 3.2% and 2.7%, respectively, increased leaching and increased nitrous oxide emissions attributable mainly to the additional urine excretion. When applications of gibberellins and nitrogen fertiliser resulted in increased herbage production but a lower herbage nitrogen concentration in late summer (scenario 3) and early spring (scenario 6), this led to small increases in nitrogen intake of 0.4% and 0.5%, respectively, compared with the control treatment. The increased excretion of nitrogen resulted in increases in annual nitrous oxide emissions from dung and urine compared with that for the control treatment of 1.8% (Table 3). The increases in excretion, leaching and nitrous oxide emissions between scenarios 3 and 4 and scenarios 6 and 7 are attributable to the increased feed and nitrogen intake.

Table 1

Treatments applied in the OVERSEER[®] model for a simplified typical Waikato dairy farm in New Zealand as scenarios to forecast the effects of adding gibberellins (GA) in early spring (August) and late summer (February) on pasture dry matter (DM) production and nitrogen (N) losses. Details of the dairy farm are described in Appendix 1.

Scenario	1	2	3	4	5	6	7
Treatment	Control	+GA	+GA	+GA	+GA	+GA	+GA
Month gibberellins applied		February	February	February	August	August	August
Pasture production (kg DM ha ⁻¹ year ⁻¹)	No change	No change	+500	+500	No change	+500	+500
N in herbage (%)	4.0	3.6	3.6	4.0	3.6	3.6	4.0
Months when 50 kg N ha ⁻¹ applied:							
August	√	√	√	√		√	√
October	√	√	√	√	√	√	√
December	√	√	√	√	√	√	√
February	√		√	√	√	√	√

Table 2

Seasonal rates of nitrogen (N) excreted in urine (kg N ha^{-1}) for treatments at the Waikato dairy farm estimated from the OVERSEER® model. The scenario numbers refer to the treatments described in Table 1, and the months when gibberellins were applied (February, late summer) and August (early spring) are shown in bold.

Scenario	1	2	3	4	5	6	7
January	53.3	53.3	53.3	53.3	53.3	53.3	53.3
February	47.1	41.7	41.7	47.1	47.1	47.1	47.1
March	49.7	49.7	65.3	64.3	49.7	49.7	49.7
April	28.8	28.8	28.8	28.8	28.8	28.8	28.8
May	20.0	20.0	20.0	20.0	20.0	20.0	20.0
June	21.2	21.2	21.2	21.2	21.2	21.2	21.2
July	36.9	36.9	36.9	36.9	36.9	36.9	36.9
August	45.2	45.2	45.2	45.2	39.9	39.9	45.2
September	39.4	39.4	39.4	39.4	39.4	53.7	53.7
October	41.8	41.8	41.8	41.8	41.8	41.8	41.8
November	52.6	52.6	52.6	52.6	52.6	52.6	52.6
December	57.2	57.2	57.2	57.2	57.2	57.2	57.2
Annual total	493	488	503	508	488	502	508

Table 3

Annual nitrogen (N) intake by grazing cattle (kg N ha^{-1}), losses of nitrogen excreted (kg N ha^{-1}), nitrogen leached (kg N ha^{-1}) and nitrous oxide emissions (kg N ha^{-1}) for treatments at the Waikato dairy farm estimated from the OVERSEER® model. Nitrous oxide emissions are calculated using emission factors from Kelliher et al. (2014) for dairy urine and dung and for reduced use of nitrogen fertiliser. The scenario numbers refer to the treatments described in Table 1 and the numbers shown in parentheses are percentage changes from the control treatment number 1.

Scenario	1	2	3	4	5	6	7
N intake	565	559 (-1.0)	567 (0.4)	579 (2.6)	560 (-0.9)	568 (0.5)	579 (2.5)
N excreted as urine	344	338 (-1.7)	350 (1.7)	355 (3.2)	339 (-1.5)	350 (1.7)	355 (3.2)
N excreted as dung	149	150 (0.7)	153 (2.7)	153 (2.7)	149 (0.0)	152 (2.0)	153 (2.7)
N leaching from urine patches	41	38 (-7.3)	42 (2.4)	42 (2.4)	37 (-9.8)	40 (-2.4)	41 (0.0)
Total farm N leaching	46	43 (-6.5)	47 (2.2)	48 (4.3)	41 (-10.9)	46 (0.0)	46 (0.0)
Nitrous oxide emissions from dung and urine	4.33	4.27 (-1.6)	4.41 (1.8)	4.47 (3.2)	4.28 (-1.3)	4.41 (1.8)	4.47 (3.2)
Nitrous oxide emissions from nitrogen fertiliser	0.96	0.72 (-25)	0.96 (0)	0.96 (0)	0.72 (-25)	0.96 (0)	0.96 (0)
Total nitrous oxide emissions	5.29	4.99 (-5.8)	5.37 (1.5)	5.43 (2.6)	5.00 (-5.6)	5.37 (1.4)	5.43 (2.6)

Reductions in annual nitrous oxide emissions compared with that for the control treatment did result when the application of gibberellins replaced addition of nitrogen fertiliser for one of the split dressings (scenarios 2 and 5). These scenarios resulted in reduced nitrogen intake associated with lower nitrogen concentrations in the herbage, reduced nitrogen excretion in urine and reduced nitrous oxide emissions from dung and urine of 1.6% and 1.3% for applications in late summer (scenario 2) and early spring (scenario 5), respectively. Across all the scenarios, the differences in change in nitrogen balance components between applications of gibberellins in late summer and early spring are small. Our findings confirm that one application of gibberellins in early spring or late summer could substitute for the need to apply nitrogen fertiliser to increase herbage production and reduce nitrous oxide emissions. When integrated to an annual basis, the effects on emissions are small. However, the reductions in emissions attributable to the substitution of one split application of nitrogen fertiliser with gibberellins in late summer (scenario 2) and in spring (scenario 5) are greater, resulting in total reduced annual nitrous oxide emissions of 5.8% and 5.6%, respectively (Table 3).

9. Discussion and insights

More recent findings confirm those from earlier studies that applications of gibberellins stimulate dry matter production in grasslands (Zaman et al., 2014b). Application of gibberellins has been shown to increase herbage growth throughout the growing season, although there is evidence that the response is less marked in the main growing season in summer (Parsons et al., 2013). Gibberellins can be used most effectively to increase rates of dry matter production in spring by enhancing the rate of stem and leaf elongation and in autumn by rapid mobilisation of stored carbohydrate reserves (Matthew et al., 2009) and reallocation of nitrogen to shoots at the expense of roots (Zaman et al., 2014b). When combined, these applications have the effect of increasing the length of the season for dry matter production. The average increase in production of 500 kg DM ha⁻¹ following one application of gibberellins reported from the analysis of pot and field experiments treated and untreated with gibberellins up until the year 2009 (Matthew et al., 2009) is reasonable, but the magnitude of the increase will depend on weather conditions and farm management practices. This increase is significant, but may be difficult to detect given the high spatial variability in biomass at paddock scales. We have adopted this increase in dry matter production in our analysis of the implications of application of gibberellins for nitrogen inputs and losses, to estimate changes in nitrous oxide emissions.

Nitrous oxide emissions from urine are much higher than those from dung. Our calculation shows that application of gibberellins within a few days after grazing can decrease nitrous oxide emissions for a single urination event by 14% compared with the emissions from untreated pasture. When one application of gibberellins is integrated to estimate the annual effect, our modelling analysis shows that the reduction in emissions was between 1% and 2%. While this is small, multiple applications of gibberellins following each grazing event could become an option to reduce emissions more substantially, as shown by Ghani et al. (2014). However, application of gibberellins should not be seen as a complete substitute for addition of nitrogen fertiliser as an approach to increasing the rate of herbage growth and dry matter production. The additional growth that occurs with gibberellin treatment alone will increase the nitrogen uptake from the soil (Edmeades, 2009; Parsons et al., 2013). Lower nitrogen in soil could further lead to losses of soil carbon since the ratio of carbon to nitrogen in soils is conserved (Schipper et al., 2010). For example, an increase in dry matter production of 500 kg ha⁻¹ following application of gibberellins with a foliage nitrogen concentration of 3.6% would lead to an uptake of 36 kg N ha⁻¹ from the soil, assuming the efficiency of mineralised nitrogen at 50%. For a constant C:N ratio of 15:1, this will result in decomposition of soil organic carbon of 540 kg C ha⁻¹. Such a change is equivalent to the average annual loss of soil carbon of 300 kg C ha⁻¹ observed over two to three decades for grazed grassland at flat sites by Schipper et al. (2010).

With the use of gibberellins there will be need to sustain mineral soil nitrogen at appropriate level using nitrogen inputs, either from added fertiliser or from nitrogen fixation by the plants, to compensate for

the nitrogen removed off-site as nitrate leaching and nitrous oxide emissions (Zaman et al., 2014b). The effect of gibberellins in promoting the proportion of legumes in mixed grass/clover swards and subsequent nitrogen fixation may alleviate some of the effect associated with drawing down of mineral nitrogen. Without mineral nitrogen being sustained, successive applications of gibberellins may lead to reduced nitrous oxide emissions at farm scales but at the expense of reduced herbage production as growth rates become limited by nitrogen supply. However, enhanced growth in the cool season (late autumn to early spring) is particularly valuable for additional animals, but is also more effective in reducing nitrous oxide emissions because this draws down the mineral nitrogen in the soil when high amounts can lead to high emissions when the soils are often cold and wet during this period (van der Weerden et al., 2014). The integrated effects of the application of gibberellins on herbage production and quality, soil nitrogen and carbon dynamics and nitrous oxide emissions require further experimental investigation. This will need to incorporate the effects of seasonality of application of gibberellins, addition of fertiliser and sward composition.

Even though the effects of gibberellins on the ratio of carbohydrates to protein (WSC:CP), metabolisable energy (ME) and nitrogen content of the herbage are small, when growth is stimulated by gibberellins relative to that for untreated controls, there are opportunities to reduce nitrous oxide emissions. It is important to consider the consequences of applying gibberellins in place of nitrogen fertiliser as a strategy to increase dry matter production. There is strong evidence that both nitrogen fertiliser and application of gibberellins increase dry matter production. This and associated reviews (Parsons et al., 2013; Shepherd and Lucci, 2013) showed that, in contrast to nitrogen fertiliser alone, application of gibberellins is not associated with an overall increase in the nitrogen content of the herbage relative to that for untreated controls. Although the increased dry matter production (and hence potentially nitrogen intake per animal) will increase for grassland systems treated with nitrogen and gibberellins, the potential losses of nitrogen to the environment will be lower with application of gibberellins alone because nitrogen intake will be lower.

Our findings highlight that the annual reduction in nitrous oxide emissions of between 5 and 6% are more attributable to the substitution of just one seasonal split application of nitrogen fertiliser by gibberellins than the reductions in urine and dung deposition. This has the added benefit that, for the purposes of the national inventory and a trading scheme, reductions in nitrous oxide emissions from reduced use of nitrogen fertiliser are easy to capture. Reductions in emissions resulting from a temporary change in the amount of nitrogen in the diet of animals are much more difficult to capture.

Acceptance of widespread use of gibberellins will be dependent on cost–benefit analysis for farmers. If the substitution of one split application of nitrogen fertiliser with gibberellins is cost effective for farmers then, in the context of farm operations, application of gibberellins should be considered as one option in a suite of improved management practices to reduce emissions (de Klein and Ledgard, 2005). A further, broader consideration is that the benefit of using gibberellins to reduce nitrous oxide emissions may be offset by the emissions from manufacturing and transport processes when life cycle analysis is incorporated.

Acknowledgements

This work was funded by the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC). We thank Geoffrey Mercer and David Wheeler for providing us with the output from the scenarios using the OVERSEER® model. We are grateful to Keith Cameron, Hong Di, Surinder Saggar, Donna Giltrap and Harry Clark for helpful comments and suggestions and to Christine Bezar for editorial improvements.

References

- Abalos, D., de Deyn, G.B., Kuyper, T.W., van Groenigen, J.W., 2014. Plant species identity surpasses species richness as a key driver of N₂O emissions from grassland. *Global Change Biol.* 20, 265–275.
- Allen, M., 2010. The effects of gibberellic acid and time of grazing on nitrogen partitioning in dairy cows grazing perennial ryegrass pastures. B. Ag. Sci. (Hons.) Dissertation, Lincoln University, Canterbury, New Zealand. 45 p.
- Ball, C.C., Parsons, A.J., Rasmussen, S., Shaw, C., Rowarth, J.S., 2012. Seasonal differences in the capacity of perennial ryegrass to respond to gibberellin explained. *Proc. N.Z. Grassland Assoc.* 74, 183–188.
- Beever, D.E., Losad, H.R., Cammell, C.B., Evans, R.T., Haines, M.J., 1986. Effect of forage species and season on nutrient digestion and supply in grazing cattle. *Brit. J. Nutr.* 56, 209–255.
- Biddiscombe, E.F., Arnold, G.W., Scurfield, G., 1962. Effects of gibberellic acid on pasture and animal production in winter. *Aust. J. Agric. Res.* 13, 400–413.
- Brenner, M.L., 1987. The role of hormones in photosynthate partitioning and seed filling. In: Davies, P.J., (Ed.), *Plant Hormones and their Role in Plant Growth and Development*. Springer, Dordrecht. pp. 474–493.
- Brian, P.W., Elson, G.W., Hemming, H.G., Radley, M., 1954. The plant-growth-promoting properties of gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*. *J. Sci. Food Agric.* 5, 602–612.
- Brown, R.H., Blaser, R.E., Fontenot, J.P., 1963. Digestibility of grasses treated with gibberellic acid. *Journal of Animal Science* 22, 1038–1042.
- Champéroux, A., 1962. Effets de la gibbérelline et de la nutrition azotée sur la corissance et le métabolisme azoté du dactyle. *Ann. Physiol. Végétale* 4, 99–114.
- Cosgrove, G.P., Edwards, G.R., 2007. Control of grazing intake. In: Rattray P.V., Brookes, I.M., Nicol, A.M. (Eds.), *Pastures and Supplements for Grazing Animals*. Occas. Publ. No. 14, N.Z. Soc. Anim. Prod., pp. 61–80.
- DairyNZ, LIC, 2012. New Zealand Dairy Statistics 2011-12. Accessed 1 September 2014 from <http://www.lic.co.nz/lic/user/File/DAIRY-STATISTICS-2011-12.pdf>.
- Davies, P.J., 1995. The plant hormones: Their nature, occurrence, and functions. In: Davies, P.J., (Ed.), *Plant Hormones: Physiology, Biochemistry and Molecular Biology*. Vol. 2. Kluwer, Dordrecht, The Netherlands, pp. 1–12.
- Denman, K.L., Brasseur, G., Chidthaisan, A., et al., 2007. Coupling between changes in the climate system and biogeochemistry. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Inter-governmental Panel on Climate Change*, Cambridge University Press.
- de Klein, C.A.M., Ledgard, S.F., 2005. Nitrous oxide emissions from New Zealand agriculture – key sources and mitigation strategies. *Nutr. Cycl. Agroecosyst.* 72, 77–85.
- Dijkstra, P., Reegen, H., Kuiper, P.J.C., 1990. Relation between relative growth rate, endogenous gibberellins, and the response to applied gibberellic acid for *Plantago major*. *Physiol. Plant.* 79, 629–634.
- Dijkstra, J., Onema, O., Van Groenigen, J.W., Spek, J.W., Van Vuuren, A.M., Bannik, A., 2013. Diet effects of urine composition of cattle and N₂O emissions. *Animal* 7 (Supplement 2), 292–302.
- Drake, S.R., Moffitt, H.R., Kupferman, E.M., 1990. Quality characteristics of ‘Bing’ and ‘Rainier’ sweet cherries treated with gibberellic acid, following fumigation with methyl bromide. *J. Food Qual.* 14, 119–125.
- Edmeades, D.C., 2009. ProGibb-SG. An independent assessment of the science. A report to NuFarm Ltd, Hamilton, New Zealand. Summary available at <http://www.progibb.com.au/node/16> (accessed 26 September 2014).
- Edwards G.R., Parsons A.J., Rasmussen, S., 2007. High sugar ryegrasses for dairy systems. In: Chapman D.F., Clark, D.A., MacMillan K.L., Nation D.P., (Eds.), *Meeting the Challenges for Pasture-based Dairying*. University of Melbourne, National Dairy Alliance, Australia, pp. 307–334.
- European Environment Agency, 2014. Why did greenhouse gas emissions decrease in the EU between 1990 and 2012? Copenhagen, 59 p.
- Finn, B.J., Nielsen, K.F., 1959. Effects of gibberellin on forage yields of six grass and legume species. *Can. J. Plant Sci.* 39, 175–182.

- Fletcher, W.W., Alcorn, J.W.S., Raymond, J.C., 1958. Effect of gibberellic acid on the nodulation of white clover (*Trifolium repens* L.) *Nature* 182, 1319–1320.
- Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W., Haywood, J., Lean, J., Lowe, D.C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M., Van Dorland, R., 2007. Changes in atmospheric constituents and in radiative forcing. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L., (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Inter-governmental Panel on Climate Change*. Cambridge University Press, pp. 129–234.
- Funnell, K.A., MacKay, B.R., Lawoko, C.R.O., 1992. Comparative effects of promalin and GA, on flowering and development of *Zantedeschia 'Galaxy'*. *Acta Hort.* 292, 173–179.
- Galbally, I.E., Meyer, M.C.P., Wang, Y.P., Smith, C.J., Weeks, I.A., 2010. Nitrous oxide emissions from a legume pasture and the influences of liming and urine addition. *Agric. Ecosyst. Environ.* 136, 262–272.
- Ghani, A., Ledgard, S., Wyatt, J., Kato, W., 2014. Agronomic assessment of gibberellic acid and cytokinin plant growth regulators with nitrogen fertiliser for increasing dry matter production and reducing the environmental footprint. *Proc. N.Z. Grassland Assoc.* 76, 177–182.
- Groenigen, J.W., van Kuikman, P.J., Groot, W.J.M., Vethof, G.L., 2005. Nitrous oxide emission from urine treated soil as influenced by urine composition and soil physical conditions. *Soil Biol. Biochem.* 37, 463–473.
- Haynes, R.J., Williams, P.H., 1993. Nutrient cycling and soil fertility in grazed pasture ecosystems. *Adv. Agron.* 49, 119–199.
- Higgs, R.J., Sheahan, A.J., Mandok, K., van Amburgh, M.E., Roche, J.R., 2013. The effect of starch-, fiber-, or sugar-based supplements on nitrogen utilization in grazing dairy cows. *J. Dairy Sci.* 96, 3857–3866.
- Kelliher, F.M., Cox, N., van der Weerden, T.J., de Klein, C.A.M., Luo, J., Cameron, K.C., Di, H.J., Giltrap, D., Rys, G., 2014. Statistical analysis of nitrous oxide emission factors from pastoral agriculture field trials conducted in New Zealand. *Environ. Poll.* 186, 63–66.
- Kiba, T., Kudo, T., Kojima, M., Sakakibara, H., 2011. Hormonal control of nitrogen acquisition: Roles of auxin, abscisic acid, and cytokinin. *J. Exp. Bot.* 62, 1399–1409.
- Kurepin, L.V., Dahal, K.P., Savitch, L.V., Singh, J., Bode, R., Ivanov, A.G., Hurry, V., Hüner, N.P.A., 2013. Role of CBFs as integrators of chloroplast redox, phytochrome and plant hormone signaling during cold acclimation. *Int. J. Mol. Sci.* 14, 12729–12763.
- Kurepin, L.V., Walton, L.J., Pharis, R.P., Emery, R.J.N., Reid, D.M., 2011. Interactions of temperature and light quality on phytohormone-mediated elongation of *Helianthus annuus* hypocotyls. *Plant Growth Regul.* 64, 147–154.
- Kurepin, L.V., Zaman, M., Pharis, R.P., 2014. Phytohormonal basis for the plant growth promoting action of naturally occurring biostimulators. *J. Sci. Food Agric.* 94, 1715–1722.
- Lester, D.C., Carter, O.G., 1970. The influence of temperature upon the effect of gibberellic acid on the growth of *Paspalum dilatatum*. *Proc. 11th Int. Grassland Congr., Surfers Paradise, Queensland, Australia*, pp. 615–618.
- Luo, J., de Klein, C.A.M., Ledgard, S.F., Saggart, S., 2010. Management options to reduce nitrous oxide emissions from intensively grazed pastures: A review. *Agric. Ecosyst. Environ.* 136, 282–291.
- Matthew, C., Hofmann, W.A., Osborne, M.A., 2009. Pasture response to gibberellins: a review and recommendations. *N.Z. J. Ag. Res.* 52, 213–225.
- McGrath, D., Murphy, P., 1976. Promotion of early grass growth using gibberellic acid. *Irish J. Agric. Res.* 15, 257–263.
- Miller, L.A., Moorby, J.M., Davies, D.R., Humphreys, M.O., Scollan, N.D., MacRae, J.C., Theodorou, M.K., 2001. Increased concentration of water soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass For. Sci.* 56, 383–389.
- Ministry for the Environment, 2013. *New Zealand's Greenhouse Gas Inventory 1990–2011*. <http://www.mfe.govt.nz/publications/climate/greenhouse-gas-inventory-2013/index.html>.
- Mitchell, K.J., 1956. The influence of light and temperature on the growth of pasture species. *Proc. 7th Int. Grassland Congr., Wellington*, pp. 58–69.
- Morgan, D.G., Mees, G.C., 1958. Gibberellic acid and the growth of crop plants. *J. Agric. Sci., Camb.* 50, 49–59.

- Müller, C., Sherlock, R.R., 2004. Nitrous oxide emissions from temperate grassland ecosystems in the Northern and Southern Hemispheres. *Glob. Biochem. Cycles* 18, GB002175 1–11.
- Parsons, A.J., Rasmussen, S., Liu, Q., Xue, H., Ball, C., Shaw, C., 2013. Plant growth – resource or strategy limited: insights from responses to gibberellin. *Grass For. Sci.* 68, 577–588.
- Percival, N.S., 1980. Cool-season growth response of kikuyu grass and ryegrass to gibberellic acid. *N.Z. J. Agric. Res.* 23, 97–102.
- Ramaswamy, V., Boucher, O., Haigh, J.D., Hauglustaine, D., Haywood, J., Myhre, G., Nakajima, T., Shi, G.Y., Solomon, S., 2001. Radiative forcing of climate change. In: Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A., (Eds.), *Climate Change 2001: The Scientific Basis. IPCC Working Group I, Third Assessment Report*. Cambridge University Press, pp. 349–416.
- Rochette, P., Janzen, H.H., 2005. Towards a revised coefficient for estimating N₂O emissions from legumes. *Nutr. Cycl. Agroecosyst.* 73, 171–179.
- Saggar, S., Bolan, N.S., Bhandral, R., Hedley, C.B., Luo, J., 2004. A review of emissions of methane, ammonia and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *N.Z. J. Agric. Res.* 47, 513–544.
- Saggar, S., Luo, H., Giltrap, D.L., Maddena, M., 2009. Nitrous oxide emissions from temperature grasslands: processes, measurements, modelling and mitigation. In: Sheldon, A.I., Barnhart, E.P. (Eds.), *Nitrous Oxide Emissions Research Progress*. Nova Science New York, pp. 1–66.
- Schipper, L.A., Parfitt, R.L., Ross, C., Baisden, W.T., Claydon, J.J., Fraser, S., 2010. Gains and losses in C and N stocks of New Zealand pasture soils depend on land use. *Agric. Ecosyst. Environ.* 139, 611–617.
- Schwechheimer, C., 2008. Understanding gibberellic acid signalling – are we there yet? *Curr. Opin Plant Biol.* 11, 9–15.
- Scurfield, G., Biddiscombe, E.F., 1959. Effects of gibberellic acid on winter pasture production. *Nature* 183, 1196–1197.
- Shepherd, M., Lucci, G.M., 2013. A review of the effect of autumn nitrogen fertiliser on pasture nitrogen concentration and an assessment of the potential effects on nitrate leaching risk. *Proc. N.Z. Grassland Assoc.* 75, 197–204.
- Tas, B., 2007. Nitrogen utilization of perennial ryegrass in dairy cows. In: Elgersma, A., Dijkstra, J., Tamminga, S., (Eds.), *Fresh Herbage for Dairy Cattle*, Springer, The Netherlands, pp. 125–140.
- Thurber, G.A., Douglas, J.R., Galston, A.W., 1958. Inhibitory effects of nodulization in dwarf beans, *Phaseolus vulgaris*. *Nature* 181, 1082–1083.
- van der Weerden, T.J., Manderson, A., Kelliher, F.M., de Klein, C.A.M., 2014. Spatial and temporal nitrous oxide emissions from dairy cattle urine deposited onto grazed pastures across New Zealand based on soil water balance modelling. *Agric. Ecosyst. Environ.* 189, 92–100.
- 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. *Proc. N. Z. Grassland Assoc.* 75, 145–150.
- van Rossum, M.H., 2013. Dry matter production, nutritive value and botanical composition of a perennial ryegrass white clover pasture applied with GA and N in successive periods in autumn and spring. *M. Ag. Sci. Thesis*, Lincoln University, Canterbury, New Zealand. 59 p.
- van Soest, P.J., 1982. *Nutritional Ecology of the Ruminant*, fourth ed. Cornell University Press, Ithaca, NY, USA.
- Wheeler, D.M., Ledgard, S.F., de Klein, C.A.M., Monaghan, R.M., Carey, P.L., McDowell, R.W., Johns, K.L., 2003. OVERSEER® Nutrient Budgets – moving towards on-farm resource accounting. *Proc. N. Z. Grassland Assoc.* 65, 191–194.
- Wheeler, D.M., Shepherd, M.A., Selbie, D.R. 2013. From stock numbers to N leaching and nitrous oxide - the progression through Overseer. In: Currie, L.D., Christensen, C.L. (Eds.), *Accurate and Efficient Use of Nutrients on Farms. Occas. Rep. 26*. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 10 p.
- Wheeler, D.M., 2014. Characteristics of pasture in: Overseer technical manual. Accessed 1 September 2014 from <http://www.overseer.org.nz/OVERSEERModel/Information/Technicalmanual.aspx>.
- Whitney, A.S., 1976. Effects of gibberellic acid on the cool season regrowth of two tropical forage grasses. *Agron. J.* 68, 365–369.

- Wittwer, S.H., Bukovac, M.J., 1957. Gibberellin and higher plants. V. Promotion of growth in grass at low temperatures. *Quart. Bull. Michigan Univ. Agric. Exp. Station* 39, 682–686.
- Woledge, J., Pearse, J.P., 1985. The effect of nitrogen fertilizer on the photosynthesis of leaves of a ryegrass sward. *Grass For. Sci.* 40, 305–309.
- Zaman, M., Ghani, A., Kurepin, L.V., Pharis, R.P., Khan, S., Smith, T.J., 2014a. Improving ryegrass-clover pasture dry matter yield and urea efficiency with gibberellic acid. *J. Sci. Food Agric.* 94, 2521–2528.
- Zaman, M., Kurepin, L.V., Catto, W., Pharis, R.P., 2014b. Enhancing crop yield with the use of N-based fertilizers co-applied with plant hormones or growth regulators. *J. Sci. Food Agric.* Published on line DOI 10.1002/jsfa.6938, 9p.

Appendix 1. Dairy farm parameters

Description of the simplified typical dairy farm at Ruakura, Hamilton, in the Waikato Region, New Zealand (latitude 37.8° S, 175.3° E, altitude 40 m above sea level) during the 2011/12 season when drought did not occur used to model the treatment effects using the OVERSEER® model. To simplify the simulations, it was assumed that all effluent, solids and liquid were exported, that there was no imported supplementary feed to the cows in pasture or in-shed, and a feed pad was not used.

Description	Units	Value
<i>Overview</i>		
Modelled pasture production	Mg DM ha ⁻¹ year ⁻¹	16.6 ^a
<i>Land</i>		
Effective area	ha	110
Topography	-	Rolling
Soil type	-	Allophanic
Soil Olsen phosphorus	mg kg ⁻¹	35
Susceptibility to pugging	-	Occasional
Herbage vegetation	-	Ryegrass/white clover
<i>Climate</i>		
Annual rainfall	mm	1138
Potential evaporation	mm year ⁻¹	817
Mean annual temperature	°C	13.8
<i>Livestock</i>		
Number of dairy cows	cows ha ⁻¹	3.0
Breed	-	Friesian Jersey
Average cow mass	kg	471
Cow replacement rate	%	23
Replacements derived from	-	Weaning
Wintering off	-	None
Lactation (calving – drying off)	-	20 July – 23 April
Lactation	days	278
Milk production	kg MS ha ⁻¹ year ⁻¹	1057
<i>Fertiliser applied (additional to nitrogen treatments) to the milking platform</i>		
Phosphorus ^a	kg P ha ⁻¹	54
Potassium	kg K ha ⁻¹	54
Sulphur	kg S ha ⁻¹	64

^a Fertiliser application had been estimated to maintain the phosphorus soil status at 35 mg kg⁻¹.