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

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Moniek van Rossum

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The effect of application of GA (GA) and nitrogen (N) fertiliser on dry matter (DM) production, botanical composition and nutritive value on a ryegrass – white clover pasture was examined in an autumn and spring trial at Lincoln, Canterbury, New Zealand. Each trial consisted of two periods. In Period 1, factorial combinations of GA (0 and 20g ProGibb SG©/ha) and N (0 and 50 kg N/ha) were applied to pastures and measurements made after 28 days. In period 2, each of the factorial combination of GA and N were splits and GA, N or nothing was applied, with measurements made after a further 28 days. In Period 1 in autumn, DM yield was not affected by application of GA but where N fertiliser was applied DM yield was greater (2152 kg DM/ha) than no N fertiliser applied (1496 kg DM/ha). GA did not affect clover, ME or CP content, but did increase stem length (68.5 vs. 60.5 mm). In period 2 in autumn DM yield was greater in GA treated than untreated pasture (875 vs. 528 kg DM/ha) but was similar to N fertilised pasture (712 kg DM/ha). GA increased clover yield above pasture treated with N or nothing (240 vs. 80 and 89 kg DM/ha) and altered many of the sward structure components including stem length, leaf length and leaf width. In period 1 in spring GA increased DM yield for mower cuts (1009 vs. 666 kg DM/ha) and quadrat cuts (1902 vs. 1653 kg DM/ha). GA caused the pasture to stand 40% taller than pasture not treated with GA (10.5 vs. 6.3 cm), and increased both ryegrass and clover content by 33 and 53% respectively. Pseudo stem length (73.1 vs. 55.1 mm) and leaf length (162.1 vs. 116.1 mm) were both increased 28 days after application of GA. N fertilised pasture had 44% more tillers than unfertilised pasture (15,815 vs. 8,875 tillers/m^2). In period 2 in spring pasture treated with GA or N increased DM yield over control pasture (1331 and 1385 vs. 1072 kg DM/ha). Ryegrass content was higher at the end of period 2 in GA and N treated pastures than control pasture (916 and 1100 vs. 799 kg DM/ha). CP was lower in GA treated pasture at the end of period 2 (18.7%) than N fertilised (20.7%) or control pasture (19.4%). In both autumn and spring, there was no interaction between effects of application of GA in period 1 and period 2 indicating no yield decrease of carryover effect. Therefore results from this study confirm findings from previous authors the GA can increase yield of ryegrass white clover pasture without affecting pasture production in spring, and that GA has no adverse effects on pasture ME or nutritive value. As increase in DM yield was achieved with minimal change in CP from GA application over both periods. GA application may provide additional feed without increasing the N content of livestock diet. In turn, this may help reduce the high levels of urinary N excreted and subsequent nitrate leaching that is associated with application of N fertiliser.

Keywords: GA, autumn, spring, yield, botanical composition, nutritive value, repeated application.

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Chapter 1
Introduction

The goal of New Zealand dairy farmers is to grow the highest quantity of good quality pasture they possibly can in order to achieve optimum animal production. This involves increasing pasture growth at times of the year when pasture growth is low (e.g. early spring and late autumn) due to temperature and water constraints. Both autumn and spring are critical to livestock production on pastoral land. Autumn is important as the pasture grown during this time can determine days in milk, and retaining livestock body condition score going into winter. Spring on the other hand is important in determining the amount of supplements required when feed supply does not meet feed demand.

Nitrogen (N) fertiliser application is the most common method used to produce more dry matter on a conventional livestock production property in New Zealand. A typical perennial ryegrass (*Lolium perenne L.*) - white clover (*Trifolium repens L.*) pasture can produce between 9 and 17t DM/ha/year with the addition of N fertiliser. During the season on a typical dairy farm up to 200 kg N/ha/year is applied, with the addition of N fertiliser leading to both an increase in the production and concentration of N in the herbage (Ball & Ryden, 1984; Carran, Ball, Theobald & Collins, 1982). This is suitable over the drier months when there is little or no rainfall, but with excess N in the system and under wet conditions, which occur in late autumn through to early spring, N becomes more loosely held in the ecosystem and more susceptible to loss, via nitrate leaching. The losses via animals in terms of urine and faeces result in accumulated nitrate in the soil profile, which is easily leached. This comes from an increase in the N concentration of the herbage leading to a higher level of N ingested via pasture (Carran et al., 1982).

On the other hand gibberellins (a group of plant growth regulators) are known to stimulate out of season pasture production and accelerated growth through reserve mobilisation in perennial ryegrass pastures (Morgan & Mees, 1958). The use of this substance alongside N fertiliser has been found to have a cumulative effect on pasture production because they complement each other. One of the most commonly used forms of gibberellin is GA. This form of gibberellin results in increased pasture growth after the initial application (Matthew, Hofmann, & Osborne, 2009), and along with some nitrogen can help to retain pasture production heading into and coming out of the low temperatures associated with winter (Davies & Thomas, 1983).

GA is known to significantly reduce the total N content in herbage (Finn & Nielsen, 1959; Matthew et al., 2009; Morgan & Mees, 1958) and increased yields of between 200-500 kg DM/ha have been reached with varying levels of GA applied (Matthew et al., 2009). Potentially, this means the use of GA may lead to a reduction in the amount of N being consumed in the herbage by the animal which in turn may lead to a reduction in the level of N excreted onto pasture.

However, to date most of the research has been based on single applications of GA, and there is inconclusive evidence on whether repeated application of GA increases or inhibits pasture production. Because GA utilises reserve N and carbon for leaf regeneration before photosynthesis can resume, a repeated application may determine the amount of reserves the plant has left for mobilisation and growth (Schnyder & De Visser, 1999).

Further, there is little data on how GA application may affect pasture quality. GA is known to cause an erect growth habit and to promote stem elongation as well as decrease the number of tillers in the sward (Blacklow & Macquire, 1971; Carran et al., 1982). This raises the question of whether GA will affect the holistic structure of the sward and achieve an increase in DM yield, which may in turn have an effect on the way livestock consume the pasture. Livestock may not eat down to the same height, and if they do, may consume more stem if sward structure is elevated. GA expands and lengthens rather than multiplies plant cells during the day (Humphries & Wheeler, 1960). This means there is only a slight increase in solutes in the plant, however there may be a higher water content and this can lead to a low solute level, which in turn results in a lower dry matter percentage so an increase in pasture growth of GA treated pasture may not be fully supported by an increase in DM of the pasture.

1.1.1 Objectives

The research objective for this study was to:

1. To determine the effect of single and repeated application of GA and N in spring and autumn on DM yield, botanical composition and nutritive value of a perennial ryegrass white clover pasture.

Chapter 2
Literature review

GA (GA) is a natural growth promotant which causes stem elongation and growth in a range of plant species (Arnold, Bennett, & Williams, 1967; Biddiscombe, Arnold, & Scurfield, 1962a; Blacklow & McGuire, 1971; P. W. Brian, Elson, Hemming, & Radley, 1954; Finn & Nielsen, 1959; Matthew et al., 2009). However, limited work has been done on the effect of repeated application of GA on pasture growth and composition. The objectives of the literature review are to explain what GA is and how it can affect different plant parts. The review will highlight previous work on the effects of GA on plant morphology, pasture production (DM yield) and pasture composition, with particular reference to perennial ryegrass where possible. Potential negative impacts of GA application on pasture growth are discussed, and the typical pasture growth response to nitrogen fertiliser (N) highlighted so that comparisons can be made between GA and N.

2.1 What is GA?

Plant growth regulators are substances that, when added in small amounts, modify the growth of plants, usually by stimulating or inhibiting part of the natural growth regulatory system (Srivastava, 2002). Gibberellins are one of four plant growth regulators that were first found to be produced by the sac fungus *Gibberella fujikuroi* and have now been found to be produced in other higher plants (Morgan & Mees, 1958; Taiz & Zeiger, 2010; Davies, 2010). Gibberellins are a large class of cyclic diterpenes or "plant hormones" that occur in all vascular plants as well as in many fungi. Over 136 known gibberellins exist yet only a few of these have intrinsic biological activity (Davies, 2010; Taiz & Zeiger, 2010). GA is one of the most common forms of Gibberellins (GA or GA_3), having been identified in 45 plants (Davies, 2010), and is the most widely used form of gibberellin in physiological studies (Matthew et al., 2009). Figure 2.1 shows the chemical composition of GA (GA_3) (Taiz & Zeiger, 2010). GA is produced via biosynthesis and its work within the plant is extremely complex. GA biosynthesis varies not only between plant species, but also in different organs within one plant species, or in response to different environmental conditions (Taiz & Zeiger, 2010). GA is produced in plant buds or stem apex and especially in leaf primordia, in the embryos of immature seeds, in fruit tissue and in roots (Paleg, 1965; Saigo & Saigo, 1983). The large amount produced in the roots is made available to the whole plant, by translocation up and down the plant axis via the plants transport systems (Paleg, 1965; Taiz & Zeiger, 2010). Evidence suggests that GA in most plants moves in a non-polar manner. The movement of GA within the plant depends on environmental stimuli and the concentration of other plant hormones (as mentioned before). For example in wheat the concentration of gibberellins was found to be highest in immature seeds, reaching 16mg/kg fresh weight, however the concentration of free gibberellins decreased rapidly when those seeds matured (Srivastava, 2002).

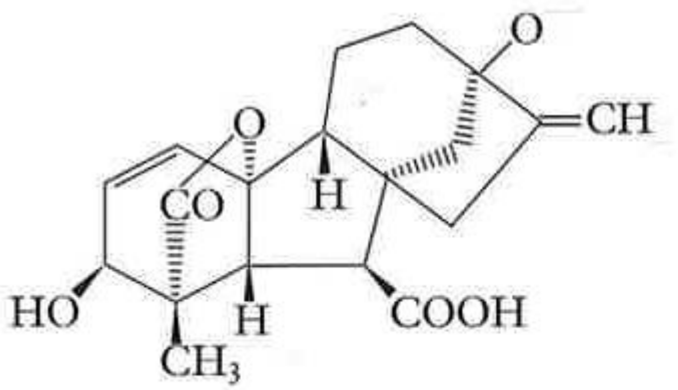

Figure 2.1: Chemical composition of GA (GA3) (Taiz & Zeiger, 2010)

Bioactive GAs serve many important roles in vascular plants and can influence a number of different physiological mechanisms, including cell elongation and division, stem, leaf and root elongation (vegetative growth) along with seed development and germination, mobilisation of food reserves, and flowering, including floral development (Davies, 2010; Tyler et al., 2004). But, plant responses to bioactive gibberellin are often complex and involve interactions between different plant organs or with other plant growth regulators including abscisic acid and auxin (Paleg, 1965). Often the different plant growth regulators network with each other to ascertain the right levels (of particular hormones) needed for growth and productivity under varying environmental conditions. These interactions (networking systems) cause complex growth and production of plants, such as mentioned above (Matthew et al., 2009; Paleg, 1965; Saigo & Saigo, 1983).

An example of one particular interaction is that of GA and auxin on the apical dominance of plants. The mechanism of GA action in the apex of a responsive plant results in increased protein synthesis, cell division, cell expansion and auxin production. Apical dominance occurs because of the increased supply of auxin resulting in a more upright growth habit than plants not subjected to the same hormones (Paleg, 1965). The effect of GA on apical dominance will be discussed in more detail later on.

2.2 Mode of action

To fully appreciate and understand how Gibberellins affect the plant phenologically, first an understanding is needed of how gibberellins work at a cellular level. Gibberellins can be thought of as “de-repressors”. That is to say gibberellins trigger, through a “signalling pathway”, the degradation of DELLA proteins. DELLA proteins are substances that exercise a growth restraint. Cross talk occurs between this gibberellin signalling pathway and other pathways which reiterate the strong interaction that occurs between the different plant hormones (Matthew et al., 2009; Taiz & Zeiger, 2010).

2.2.1.1 Level of GA within the plant

Bioactive gibberellin concentration varies between plants in the range of $10^{-11} – 10^{-9}$ g/g fresh weight; however this is dependent on plant tissue type and plant species (Davies, 2010). Gibberellins play an important role in mediating the effects of environmental stimuli on plant development because they are sensitive to both light and temperature (Paleg, 1965). Applied to pasture there is a variable growth response due to the level of bioactive GA within the plant, plants ability to access nutrients in soil and the plants stage of development, and finally whether the plant is reproductive or not. Responses vary between individual plants and plant species (Taiz & Zeiger, 2010). However, generally observed responses include out of season growth or accelerated growth of pasture which has been stimulated by reserve mobilisation, leaf and stem elongation, and the promotion of flowering via GA (Arnold et al., 1967; Biddiscombe et al., 1962a; Blacklow & McGuire, 1971; Finn & Nielsen, 1959; Fletcher, Alcorn, & Raymond, 1959; Matthew et al., 2009; McGrath & Murphy, 1976; Morgan & Mees, 1958a).

2.2.1.2 Temperature and GA response

Environmental factors, especially temperature and light are known to affect GA levels and/or the sensitivity of the plant response to GAs (P. J. Davies, 2010). Light and temperature can have profound effects on GA metabolism and GA response. In many cases the environment can alter the metabolism of, or response to, other hormones in addition to GAs. In an experiment done with tulips, it was found that decreased temperature resulted in a decreased level of free gibberellin in favour of the less active bound form of gibberellin (Blacklow & McGuire, 1971).

It is thought that gibberellin biosynthesis is actually partially controlled *in vivo* by the phytochrome system. However there are a large amount of other phytochrome responses involved. The level of endogenous GA depends on photoperiod responses (plants response to red and far red light), and because photo Period responses differ between plant species (C3 versus C4 plants) the day length required by the plant to grow and the interaction with the level of endogenous GA determines speed of relative growth (Paleg, 1965). An example of the way the environment can affect the interaction of different plant hormones and the response of the plant to an altered gibberellin level is the effect of temperature on plant architecture. Plant architecture is regulated by temperature which appears to involve the complex interaction of multiple hormone signalling networks. GA (GA), salicylic acid (SA) and cytokine have been found to be the main plant growth regulators which determine the rate of plant growth during a Period of chilling (Patel & Franklin, 2009). Temperature also regulates growth and development throughout the lifecycle of plants. In many plant species, a prolonged Period of cold (stratification) is required to promote germination through stimulation of GA biosynthesis (Patel & Franklin, 2009).

Even in temperature below 0° C, barley plants have been found to have a growth response to GA application. One study found that application of GA under low temperatures resulted in a reduction of the barley's base temperature, inflection point and activation energy needed for stem, internode and leaf elongation. This resulted in plants being able to sustain growth in terms of elongation and leaf expansion under low suboptimal conditions because the leaf extension zone became more sensitive to lower temperature (Farrell, Ougham, & Tomos, 2006).   On the other hand at high temperatures (28 – 29°C), significant elongation of plant stems, elevated leaves (hyponasty), reduced biomass and accelerated flowering occurs (Patel & Franklin, 2009). This is thought to be because the increased temperature can lead to an increase in the level of free gibberellins (Blacklow & McGuire, 1971).

In terms of frost susceptibility, it has been proposed that GA treated pastures (in this case Phalaris and subterranean clover) are more susceptible to frost damage because GA application creates a more erect growth habit of the pasture plants, making it easier for frost to settle into the open canopy. This means that precaution should be taken when applying GA when frosts may occur, though in general grasses are more tolerant of frosts than either of the species used in this trial. Recovery time following a frost needs to be taken into account before exogenous GA is applied, and this makes the incidence of frosts an important consideration when applying GA to pasture in late autumn and early spring (Biddiscombe, Arnold, & Scurfield, 1962b).

2.2.1.3 Day length and GA response

It has been speculated that under long photoperiod the amount of gibberellin in a plant increases; this leads to a response to the photoperiod because of the increase in endogenous GA, which may in turn lead to an increased growth response (Blacklow & McGuire, 1971). Some researchers have found positive growth responses when applying GA at low concentration (Between 8 - 70g GA/ha) especially during cool seasons, for example autumn, winter and early spring, and that GA could have potential for the off season production of grass (Leben, Alder, & Chichuk, 1959; McGrath & Murphy, 1976; Whitney, Green, & Younge, 1973). However, when Matthew et al. (2009) compared and analysed the results of previous studies he found that there was no conclusive evidence of a particular season when swards were more responsive to application of exogenous GA. One of the studies done looking into GA and day length found that perennial ryegrass requires both vernalisation and long day exposure to flower. Day length; for example long or short days, increase GA content, whereas vernalisation is required for the action of the GA produced (MacMillan, Blundell, & King, 2005).

When exposed to eight weeks of vernalisation at 8 ^{o}C and then two long days (48 hours made up of two 8 hour Periods of sunlight and two 16 hour Periods of low intensity incandescent lamps), a 4-fold increase in the level of endogenous GAs in the leaf and shoot of the plant was created (83.45ng GA/g dry weight produced by the long day vernalized plants compared with 20.73ng GA/g dry weight produced by shoot material in long day non vernalized plants (MacMillan et al., 2005). In all cases the majority of GA produced was produced in the shoot and less so in the leaf blades. In the long day vernalized plants, for example, the shoots contained 73.9ng GA/g dry weight whereas the leaf blades only contained 9.55ng GA/g dry weight (MacMillan et al., 2005). In regards to flowering, GA application then replaced the need for long days but did not replace the need for vernalisation. GA signalling is independent of vernalisation and that apparently impacts on unrelated processes (MacMillan et al., 2005).

In contrast to this, a recent study (Parsons et al., 2013) found that the difference in responses to GA application to perennial ryegrass between summer (small increase in yield above 6 cm) and winter (large increase in yield above 6 cm) derived plants was not due to the summer derived plants being measured at 8 hours day length and the winter derived plants being measured at 14 hours day length. However, in support of the above evidence, it was proposed that the differences in the growth response of the plants to exogenous GA application is not due to ‘current’ growing conditions (the growing conditions that the plants were subjected to during the experiment), but at the developmental state at which the GA was applied (winter ‘short day’ plants versus summer ‘long day’ plants). Temperature was also not a factor in the yield response by summer and winter derived plants subjected to exogenous GA application (Parsons et al., 2013).

2.3 GA response among plant parts

2.3.1.1 Seeds and germination

Seeds of many plants store large quantities of reserve foods such as starch, protein, and lipids which are hydrolysed following seed germination to provide energy and carbon skeletons as building blocks for growth of the young seedling. In cereal grains, gibberellins induce the *de novo* synthesis and/or activation of several different enzymes for hydrolysis of storage products which leads to germination (Davies, 2010). Seed germination is one of the main processes affected by gibberellins. Seeds are held in a dormant condition by the presence of growth inhibitors. The influence of the inhibitors must be removed before growth and germination can occur (Saigo & Saigo, 1983). Within a dormant seed is bioactive gibberellin and abscisic acid which act in an antagonistic way. The relative levels of the two hormones mentioned determine the level of dormancy a seed has (Taiz & Zeiger, 2010).

In cold temperatures, bioactive gibberellins are likely to be abundant which will then end dormancy and promote germination to occur. During germination gibberellins induce the synthesis of hydrolytic enzymes. These enzymes degrade the stored food reserves accumulated in the endosperm or embryo as the seed matures (Saigo & Saigo, 1983). This degradation of carbohydrates and storage proteins provides nourishment and energy to support seedling growth (Taiz & Zeiger, 2010). However this procedure does not encompass all plant species. In an experiment using white clover (*Trifolium repens*), exogenous application of GA had no effect on germination, the growth of rhizobia or on nodule formation (Stuart & Cathey, 1961).

There is limited information on GA and perennial ryegrass seed germination. Most experiments that apply exogenous GA to perennial ryegrass pastures use pastures that are at least one year old. With the information that is available on GA and seed germination we can hypothesis that GA will stimulate perennial ryegrass seeds to germinate. This is based on the fact that other perennial plants including *Themeda australis*, *Chrysopogon fallax*, and, *Sorghum plumosum*, have been found to germinate with exogenous GA application (Mott, 1978).

2.3.1.2 Root biomass

Root growth in response to gibberellin application varies among plants and plant species no matter the application technique (as a foliar spray, or when plants roots are soaked in a solution). In some species application of gibberellin does not affect root growth at all (Brian, 1959; Fletcher & Martin, 1962; Kato, 1958). The size of the response (if any response at all), is determined by the concentration of GA within the plant itself, the concentration of exogenous GA applied to the plant, the stage of growth the plant is in, and the timing of the application of exogenous GA (Brian et al., 1954; Finn & Nielsen, 1959; Parsons et al., 2013; Tien et al., 1979). Under some circumstances, with some plant species, treatment with GA does not stimulate growth of intact roots, though some root sections do respond by increased (P. W. Brian et al., 1954) or suppressed growth.

A glasshouse trial carried out in Canada found that with a foliar application of potassium salt GA at 0, 74, 148, 296 or 593 g /ha to *Lolium perenne* and *Trifolium repens* caused root mass to decrease with increasing rates of GA applied 60 days post application. *Lolium perenne* roots decreased from 2.49g DM/ pot at 74g GA/ha to 1.60g DM/ pot at 593g GA/ha. An interesting point to note from the study was that *Phleum pratense* (Timothy), *Poa pratensis* (Kentucky bluegrass) and *Lolium perenne* (Perennial ryegrass) showed a consistent root yield depression with increasing rates of GA applied. We can theorize that in terms of root mass, perennial ryegrass and timothy are more sensitive to GA application because they were more adversely affected than Kentucky bluegrass (Finn & Nielsen, 1959). This result was further supported by evidence where a variety of grasses including perennial ryegrass had suppressed root growth when GA was applied externally (Blacklow & McGuire, 1971).

Clover root mass reacts in a similar fashion as perennial ryegrass to exogenous GA application. In the same study as mentioned above, Ladino clover root weight decreased from 1.03g DM/L at 74g GA/ha to 0.71g DM/L at 593g GA/ha (Finn & Nielsen, 1959). In many cases, the presence or absence of N (in plant and soil solution) as well as the concentration of N has a significant impact on root response. Under low concentration of gibberellins (24g GA/ha), root growth was reduced in cocksfoot when N was limiting, but at a higher concentration (36g GA/ha) root mass was found to increase because N was not limiting (Champéroux, 1962). In a recent study it was found that N supply determined the response of plants taken from the field in winter. Plants subjected to high N supply without GA application tended to have a reduction in root mass, however plants subjected to low N supply conditions with an exogenous application of GA had an increased root mass that was 81% greater than control plants 4 weeks post application (Parsons et al., 2013).

To cause a root response to occur in many cases GA must interact with other plant hormones especially auxin, as auxin has the ability to suppress root growth while GA is thought to promote root extension and development (Brian, 1959; Fu & Harberd, 2003). . The plants shoots and roots are generally competing for the same resources for growth and development, so upsetting the equilibrium of the two hormones by applying exogenous GA is more than likely to cause a growth response of some sort. One experiment found that when GA was applied to the tip of pea, bean and citrus plants, stems extended while rooting was unaffected. However, when interacting with another hormone, in this case auxin, GA managed to promote stem extension and increase root number (Stuart & Cathey, 1961).

The rhizosphere of various grass species contains a number of different nitrogen fixing bacteria, which influence the growth and morphology of the grass plants. The products of a particular nitrogen fixing bacterium (*Azospirillum brasilense*) were investigated in study done in Florida, USA. A small but biologically significant amount of gibberellin was found to be produced by *Azospirillum brasilense.* The gibberellin was used to investigate the morphology of pearl millet roots. When applied at a low concentration (0.005 µg/ml) gibberellin was found to increase the production of lateral roots, and a combination of auxin, gibberellin and kinetin produced changes in root morphology of pearl millet such as increased lateral root number and dense covering of the lateral roots with root hairs. The response lasted up to 6 weeks (Tien et al., 1979). Though this may not be the case for all plants, excised tomato roots showed a similar response (Tien et al., 1979) but the opposite was true for wheat plants, as GA was found to stimulate shoot production while inhibiting root production (Brian et al., 1954).

In contrast, when applied to white clover gibberellins were found to reverse the normal diageotrophic response of stolons so that they become negatively geotropic and began growing almost vertically, rather than in their usual horizontal fashion (Fletcher & Martin, 1962). The authors felt this particular event would reduce root formation as the stolons were no longer running along the ground. Nickerson (1959) also found that adventitious root formation can be stimulated by GA in grasses, but this is dependent upon concentration within the plant and the plant species that are involved.

Many authors concluded that the morphological changes of the roots caused by GA application had a direct impact on growth and development of the vegetative (above ground) plant material. It was said that the small morphological changes might lead to greater rates of nutrient absorption, and in turn increased plant growth. The contrasting results on root production in response to GA application is thought to be due to an interactive response to

GA, as shoots and roots are both competing for the same resource. A further experiment revealed that fresh and dry weight measurements of roots showed that rapid and prolonged stem elongation of treated plants was associated with lower root growth (Marth, Audia, & Mitchell, 1956). This could be due to the use of the root reserves/ root carbohydrates, as it has been proposed that part of the energy and structural material for the increased foliar growth in the winter that was promoted by GA may have been derived from plant reserves (Blacklow & McGuire, 1971).

The most characteristic effects of GA on shoot growth are increased inter-node extension, increased leaf-growth and enhanced apical dominance (Brian, 1959). Many studies indicate that stem elongation and leaf expansion often occurs in plants in response to gibberellin application promoting internode elongation. This has been found to occur particularly in genetically dwarf mutants, rosette species and various members within the grass family (Taiz & Zeiger, 2010). Growth responses can be identified in plants as early as four days post application. When GA was applied to roots the relative growth rates of both C3 and C4 plants including wheat, barley, oats, corn, sorghum, millet, mungbean, squash, capsicum, pigweed, kochia, gomphrena were increased as early as four days post application (Tsai & Arteca, 1985).

An increased final leaf length comes from both an increase in cell number and the length of individual cells. When applied, gibberellins did not change the osmotic pressure or turgor pressure but there was a change in the cell wall properties which were measured both *in vitro* and *in vivo,* potentially increasing the fibre content of the plant (Farrell et al., 2006). This elongation response of the stem and leaf of some plant species, especially biennials including chicory and some brassicas, but not in perennial ryegrass to exogenous gibberellin application is accompanied by a rapid extension of the flowering spike/pedicel known as "bolting" (Blacklow & McGuire, 1971). Gibberellins act in the same way as increased day length or cold exposure to stimulate this "bolting" response to stimulate flowering (Blacklow & MacGuire, 1971; Davies, 2010; Matthew et al., 2009).

In both glasshouse (Finn & Nielsen, 1959; Parsons et al., 2013) and field studies (McGrath & Murphy, 1976) comparing grass species responses to foliar applications of GA, perennial ryegrass has been found to be one of the least responsive grasses. Kentucky bluegrass is more responsive to gibberellin than perennial ryegrass (0.82 and 0.22g/pot response respectively) (Finn & Nielsen, 1959). This may be due to their conservative growth habit which allows for continued growth and survival over many years, seasons and varying environmental conditions (Parsons et al., 2013). While tropical or annual grass species whose growth habit may be manipulated more easily.

One point of interest is the gibberellin application rate at which yield is reduced by further rate increase. Different pasture species reach this level at different rates. For example, this occurred for perennial ryegrass at 60 -120 g/ha gibberellin, but in Kentucky bluegrass and timothy this was not reached until 300-600 g/ha gibberellin (Finn & Nielsen, 1959). Compared to grasses legumes seem to be more sensitive to GA application and are able to respond to very high levels of externally applied GA, with Ladino clover having the highest yield at the highest rate of GA applied (593g GA/ha) (Finn & Nielsen, 1959). But, this response is only evident in the first harvest Period which for this trial was 30 days post application. When the second cut was done 60 days post application grass and legumes followed a similar trend of yield depression at increasing rates of GA (Finn & Nielsen, 1959).

2.3.1.3 GA and shoot: root ratio

The shoot to root ratio is influenced by many factors. The kind and magnitude of these effects depend largely upon the environmental conditions to which the plant is exposed, and will vary between plant species. In one trial it was found that the effect of GA on the distribution of assimilates was indicated by the changes of the shoot/root ratio. The shoot/root ratio was increased from 2.3 to 3.1 for the northern European Tall fescue variety and reduced from 3.5 to 3.0 for the Mediterranean Tall fescue variety, though this result was not significant (Blacklow & McGuire, 1971). It is thought that the influence of GA on root production may, perhaps, be compared to that produced by excess nitrogen (Juska, 1959).

2.3.2 GA and Leaf growth

GA has been found to have a significant effect on both the growth of the lamina and other plant phytometers in both legumes and grasses. In pinto bean, it was found that primary leaves of plants treated with the low GA application rate (0.1p.p.m GA) developed longer petioles and the leaves of the plants expanded more rapidly than the untreated plants. Seven days after being treated with GA the leaf blades of young pinto bean plants (which are very sensitive to GA) had 24-56% greater leaf blade area than control plants (Marth et al., 1956). Similarly ladino clover was found to have increased petiole and internode length with a slight increase in leaf size (Finn & Nielsen, 1959).

2.3.3 GA and stem development

When perennial ryegrass is treated with GA it has been found that stem elongation caused by the gibberellin treatment occurs before the plant becomes reproductive and forms a flower primordial (Davies, 1973). However this response is also temperature dependant as in situations where perennial ryegrass is subjected to low temperature vernalisation, the opposite is true (Davies, 1973). There was no significant effect found with stem length (known in this experiment as stubble) when GA was applied to a perennial ryegrass pasture taken from the field in winter (Parsons et al., 2013). However in a high N environment stubble length was greater for plants in high N environment compared to plants in a low N environment (Parsons et al., 2013). It has been found that the greatest stem elongation resulted when the chemical GA was applied to stems that had just begun to elongate (pre reproduction) (Marth et al., 1956). There was also an obvious effect on the lengthening of the internode length, including the internodes that were above the treated area of the plant (Marth et al., 1956). It is thought that along with N and C which act as signals to meristem activity within perennial plants; GA has a significant impact on meristematic activity (Parsons et al., 2013). The authors proposed the idea that exogenous application of GA may manipulate the way meristem signals are interpreted and in turn this may alter the prompts affecting meristem activity (Parsons et al., 2013).

Though stem elongation is mainly found in plants before they become reproductive; GA has been found to cause some plants to become reproductive. If this happens in pasture a rapid deterioration of the leafiness of the regrowth can be noted because of the increased apical dominance, stem development and increased internode length (Davies, 1973). This affect may also cause a response in tiller density.

2.3.4 GA and tiller or stolon density

Reproductive development interacts strongly with grass growth and development. Once apices initiate, growth is stimulated, but the numbers of tillers can then decline and plants become more vulnerable to damage from grazing, especially during stem elongation (Kemp & Culvenor, 1994). Tiller number per unit area in an established sward may fluctuate seasonally but is often fairly stable during Periods of uninterrupted growth (Silsbury, 1970). When this occurs leaf production will be restricted to a constant number of sites and at each of these sites growth will be constant. Indeed it can be proposed as a general thesis that a grass sward will, at least during the short term in a normal season, show a constant growth rate once complete light interception has been achieved. In a non-treated perennial ryegrass sward the average stem tiller density was 2400 – 3000 tillers/m^2 (Davies, 1973).

GA application has been found to have a marked effect on the growing plant. GA application increased both petiole length and individual leaf area and decreased the total leaf number, stolon number and stolon length per plant (Fletcher & Martin, 1962). In this experiment, it was found that the petiole length was almost doubled, and leaf area was increased 2-3 fold to compensate for the decreased number of leaves. The reduction in leaf number (decrease by about half) came about because of the reduction of stolon number and length (also decreased by about half) as there were less leaves produced along the shortened stolon (Fletcher & Martin, 1962). The increase in leaf size was accompanied by a change in leaflet shape, with the originally oval shaped leaflets becoming more elongated.

In terms of tiller density and leaf appearance gibberellin application has been found to reduce tiller number per plant from 93 to 63 in a European tall fescue (Blacklow & MacGuire, 1971). In this trial it was suggested that this might have been due to gibberellin related increase in endogenous auxin, which therefore increased apical dominance. Because of apical dominance occurring, lateral outgrowths were also found to be reduced by GA application in many plant varieties including perennial ryegrass (Paleg, 1965). Other trials have found similar effects and gibberellin induced reduction in tillering before seed head development (Hampton & Hebblethwaite, 1984) but has also been found to reduce seed head numbers in ryegrass and phalaris which consequently reduced total seed crop production. Other observations of decreased ground cover or an apparent increase in apical dominance following gibberellin use include Kentucky blue grass and kikuyu (Matthew et al., 2009). Along with a reduction in leaf appearance and tiller number GA can also reduce chlorophyll levels.

2.3.5 GA and forage composition

The botanical composition and population of grazing land, along with the morphology and structure of the sward affect the amount of herbage grown and consumed (Sanderson et al., 2004). GA (as stated above) affects the structure of the sward so may affect the amount of herbage that is grown and consumed. GA application to pastures generally leads to a yellowing of foliage particularly found to last up to two weeks in grasses, but not apparent in legumes (Finn & Nielsen, 1959). This effect is not as noticeable when GA is applied with N (Matthew et al., 2009; McGrath & Murphy, 1976). Although GA increases the amount of foliage, it fails to increase chlorophyll synthesis by a corresponding amount, which results in chlorosis (Blacklow & McGuire, 1971). Chlorosis is the yellowing or whitening of normally green plant tissue because of a decreased amount of chlorophyll, often as a result of disease or nutrient deficiency. A reduction of 50% chlorophyll reduction was found in the foliage of tall fescue following GA application (Blacklow & MacGuire, 1971) and GA was also found to reduce the chlorophyll content (expressed on an area basis) by 20.0, 13.9, 20.9, 17.1, 11.9 and 28.0% in barley, squash, pepper, sorghum, pigweed and kochia respectively, while that of oat, wheat, mung bean, corn, millet and gomphrena remained unchanged (D. S. Tsai & R. N. Arteca, 1985). In an Irish field study it was suspected that GA caused carbohydrate and starch levels to drop directly after spraying, but that by the time the pasture was harvested and analysed the carbohydrate and starch levels had returned to normal (McGrath & Murphy, 1976).

Early gibberellin studies have found that there is generally a reduction in crude protein following GA application, and later research stated that a reduction in protein content is one of the most consistent effects of GA application on chemical composition of grass (Brown, Blaser, & Fontenot, 1963). However this reduction was usually offset by increased yields, and resulted in an increase in per hectare crude protein yield (Finn & Nielsen, 1959; Matthew et al., 2009; Morgan & Mees, 1958b). In phalaris plants, the nitrogen content (%) was found to be significantly reduced ($p<0.01$), from 4.25 to 3.66 at harvest 1, 5.37 to 4.13 at harvest 2 and 4.57 to 4.20 at harvest 3 with GA application compared to control (Scurfield & Biddiscombe, 1959). Other research supports the fact that there can be a decrease in %N, but this is often not significant and is only a short term effect (Murphy & McGrath, 1978). Despite the reduction in chlorophyll content, increases in photosynthetic activity have been reported in GA treated plants because of gibberellin induced apical dominance, and more upright leaf orientation which contributes to an improved canopy light interception (Matthew et al., 2009). For example when photosynthetic rates were expressed per mg of chlorophyll, it showed that GA could stimulate photosynthesis in barley, squash, pepper, sorghum, millet, pigweed and korchia by 20.4, 20.6, 16.5, 17.4, 10.4, 24.2, and 29.4% while there was no effect in oat, wheat, mung bean, corn and gomphrena (Tsai & Arteca, 1985). This has lead researchers to the conclusion that GA treatment leads to better utilisation of plant available N (Matthew et al., 2009). This result does not however extend to clover, as N content of clover remained unchanged throughout the experiment (Scurfield & Biddiscombe, 1959). In an Irish study on a variety of pastures including perennial ryegrass and clover, a 2% reduction in N content was found. Chlorosis of the pasture was also observed, however this had disappeared by the time of the harvest (McGrath & Murphy, 1976).

The change of pastoral N content is determined by the relative change in DM yield when GA has been applied. The total N content was found to be greater for the plots treated with GA compared with the control plots with two varieties of Tall fescue, a variety of Phalaris and subterranean clover due to increased growth with GA application (Blacklow & McGuire, 1971; Scurfield & Biddiscombe, 1959). The total plant chlorophyll content was found to increase from 23.1 to 31.6 mg for the northern European tall fescue and 21.8 to 24.6 mg for the Mediterranean tall fescue following GA application (Blacklow & McGuire, 1971).

In one set of experiments it was found that when dry matter digestibility was significantly reduced, there was also a reduction in protein and an increase in nitrogen free extract content (Brown et al., 1963). Forage nutritive value is not significantly reduced by GA application in all pastoral experiments using GA as the growth promotant. For example in a field experiment done between 1975 and 1976 GA did not cause a change of pasture mineral, nitrogen or carbohydrate content (McGrath & Murphy, 1976). However crude fibre content was increased after 21 days in Australian native grasses, cocksfoot and Kentucky 31 fescue at an application rate of 10 or 20ppm, after 21 days (Brown et al., 1963).

WSC (water soluble carbohydrate) was also found to increase by 4.35% with GA application in northern European and Mediterranean varieties of tall fescue in a winter growth experiment (Blacklow & McGuire, 1971). The highest concentration of WSC was found in the stems, followed by the roots and the leaves, and GA increased the concentration of WSC in all regions of the plants. Most of this increase was due to an increase in the concentration of sucrose and to a lesser extent fructosans (Blacklow & McGuire, 1971).

Despite this, in a New Zealand study there was no effect of GA application on ME content of a perennial ryegrass-white clover pasture as measured by NIRS (Matthew et al., 2009). Biddiscombe et al (1962) noted that pasture quality declined with GA application, but did not affect LW gain as there was more forage available actually lead to increased live weight of grazing sheep despite the reduction in nutritive value (Biddiscombe et al., 1962b). Though GA does not seem to immediately impact on the live weight gain of livestock; after multiple applications and grazing’s, pasture may become unpalatable to livestock due to increased stemminess of the sward which causes the sward to be less completely consumed by grazing animals (Whitney, 1976).

2.3.6 GA and pasture DM production

Research from 1957 to the present time has found that an increase in sward height occurs within 3-4 weeks of GA application (Arnold et al., 1967; Blacklow & McGuire, 1971; P. Brian, 1959; Finn & Nielsen, 1959; Leben et al., 1959; Marth et al., 1956; Matthew et al., 2009; McGrath & Murphy, 1976; Morgan & Mees, 1958a; Parsons et al., 2013; Scott, 1959; Scurfield & Biddiscombe, 1959; Whitney, 1976). However, this does not necessarily mean that DM yield will increase. Matthew et al (2009) found that GA application increased herbage height as measured by the rising plate meter and mower yield, but no yield increase was detected with cuts to ground level. In the first trial, instead of a pre GA application trim with the mower, animals were allowed to graze across the pasture and so distributed urine and faecal material onto the trial site which may have interfered with the results, and meant that despite an increase in sward height (as measured by the rising plate meter) there was no increase in yield (Matthew et al., 2009). This supported evidence from McGrath and Murphy (1976) who found that although pastures including perennial ryegrass, timothy, tall fescue and small amounts of clover; increased in DM yield with GA application the subsequent growth was not accompanied by a change in the dry matter content.

The yield response of pasture to GA application is usually significant. In a study carried out in the UK on perennial ryegrass, cocksfoot and timothy based pastures achieved a DM production of 70 – 980 kg DM/ha compared with control pastures when a rate of 141g GA/ha was applied (Morgan & Mees, 1958b). In a Canadian study increased dry matter production of 60 – 210 kg DM/ha for perennial ryegrass, and 520-1300 kg DM/ha for white clover was achieved when 75, 150, 300 and 600 g/ha of GA was applied (Finn & Nielsen, 1959). An Irish study found an increase of 398 kg DM/ha could be achieved by applying 70g/ha of GA (Clerc, 1976). In 1959 a New Zealand study also found an increase in dry matter produced of 170 kg DM/ha on a perennial ryegrass dominant mixed sward with an application rate of 63g GA/ha (Scott, 1959). In comparison the trial done in 2009 on a ryegrass white clover pasture found that with a low application rate of 8 g GA/ha gave a response of 200 kg DM/ha (Matthew et al., 2009). This yield increase was dependant on a pre-GA application trim and, it was stated that scientists should be careful when measuring GA responses in field as proposed responses to GA application have been low which means a low sampling error is essential. One way of doing this would be to assess yield using an adequate amount of quadrat cuts that represents a similar sampling error to that of a lawn mower (Matthew et al., 2009).

Growth stage of the pasture could potentially have an impact on GA application and DM yield response, as GA was found to be less effective in increasing DM production in two tropical forage grasses once the sward entered the ‘boom’ regrowth stage of the regrowth curve (Whitney, 1976). Age of pasture can also have an effect on the size of the growth response as it has been found that an old impoverished pastures have a greater relative response after multiple applications, compared with young pastures (50 and 460kgDM/ha respectively), which are thought to be drawing on root reserves (McGrath & Murphy, 1976).

A common side effect of gibberellin application to pasture in historical experiments is a yield depression following the initial response. This yield depression has been found in both pot and field trials, and is generally equal to that of the yield increase obtained at the first cut or harvest Period. Finn and Nielsen (1959) found an average yield depression of 0.07 g/pot and 1.39 g/pot across four rates of GA applied (74, 148, 296 and 593g GA/ha) for *Lolium perenne* and *Trifolium repens* respectively. In support of this, two field experiments found similar yield decreases at the second harvest after a single GA application of 290 kg DM/ha and 90kg DM/ha 98 and 105 days after GA was applied (McGrath & Murphy, 1976; Scott, 1959). Both of these experiments were done in late winter early spring. In most cases it was found that whatever increase happened in the first cut, was equal to the depression that occurred in the second cut (which cancelled out the increase in DM yield due to GA application (Finn & Nielsen, 1959; McGrath & Murphy, 1976; Morgan & Mees, 1958a)). Yield depression caused by GA can be reduced by lightly grazing pastures to allow reserve accumulation before GA is applied. In support of this Whitney (1976) discovered that growth depression/ retardation occurred in harvested plots; but not in plots that were left unclipped, or were defoliated two weeks pre GA application.

GA yield depression in winter of a phalaris and sub clover pasture has also been shown to affect live weight gain of livestock, as pregnant ewes were found to be lighter on gibberellin treated plots compared with control plots because of the temporary decline in pasture growth after the initial Period of stimulation (Biddiscombe and Arnold (1963). These statistics on yield depression do however come from extremely high GA application rates which in some cases have been applied more than once. These application rates are much higher than currently recommended (8g GA/ha) and at the lowest rate of application (74g GA/ha) yield depression was eliminated or reversed (Finn & Nielsen, 1959). Because of increased erectness and decreased basal tillering of the gibberellins treated plants, a more severe defoliation would occur for any given defoliation height compared to untreated plants and this would lead to decreased relative growth rate of treated plants following defoliation (Matthew et al., 2009). Also winter growth response driven by plant “reserve” utilisation is usually followed by a yield depression (particularly in younger pastures, <2 years old), and this yield depression could potentially reduce herbage accumulation potential in spring (Matthew et al., 2009).

2.3.7 GA and pasture botanical composition

The way GA affects dry matter production and growth of individual pasture species is one thing, but the way in which GA affects the botanical composition of the pasture is another. In pot trials a deferential response has been found between pasture species (Morgan & Mees, 1958). Therefore when GA is applied to a pasture containing more than one species, you might expect some species to respond more than others, and that GA will therefore affect botanical composition of the pasture (refer to van Rossum, Bryant & Edwards, 2013). In turn these changes may affect livestock performance or N cycling depending on the changes in composition.

If you look closely at a pasture you will see that there is a complex structure of temporal and spatial distribution of both species and species richness in pastures (Sanderson et al., 2004). So the botanical composition and population of grazing land, along with the morphology and structure of the sward affect the amount of herbage grown and consumed (Sanderson et al., 2004). Along with this the above ground plant composition can strongly affect nutrient cycling rates in pastures, such as with the use of legumes (white clover) to increase soil nitrogen availability because of N fixation (Sanderson et al., 2004). Another example is the above ground plant diversity and its effect on nutrient cycling through microbial decomposition of plant litter. It is thought that plant litters that differ in quality (e.g. N concentration) may interact and complete decomposition at a different rate compared with a pasture with a single pasture species (Sanderson et al., 2004).

In an Irish study done on a variety of pasture species including perennial ryegrass, poa travailis, poa annua, clover agrostis tenuis, Canadian timothy and tall fescue GA applied at a rate of 70g GA/ha did not alter pasture composition. Though the authors found that perennial ryegrass became more competitive across the different treatments (McGrath & Murphy, 1976). So keeping this in mind it was interesting to discover that GA did not affect the botanical composition of all pasture types. In a phalaris and subterranean clover pasture it was found that GA significantly reduced (0.05) clover content between 8 and 12 weeks (harvest 2 and 3), which was accentuated by heavy grazing as the sheep ate most of the erect clover and after that they kept it closely defoliated. This lead to decreased production of clover in the spring Period which allowed annual grasses to emerge between the clover and phalaris plants and grow at a vigorous rate (Biddiscombe et al., 1962a). The scientists thought that the decreased level of plant production of clover may have also led to decreased seed production.

In another experiment there was no change in the pasture composition when treated with GA (10 or 70g/ha) in perennial ryegrass white clover pasture over 8 weeks (McGrath & Murphy, 1976). However the contribution of perennial ryegrass was found to have increased in both treated and control plots since the previous autumn and accounted for more than 99% of the new pasture, so this could potentially have masked any significant changes that may have been found in clover content. To support this statement an experiment found clover percentage decreased at both harvest 3 and 4 following GA treatment (Scott, 1959). Legumes have been found to be more sensitive to GA application than grasses, especially at very high rates (295 to 590g GA/ha). Recent studies contradict these results. It was found that 4 weeks post GA application perennial ryegrass white clover pastures contained 96% more clover than control plots (van Rossum et al., 2013). Allen (2010) found that clover content was 18.7% higher in GA treated pasture compared with pasture treated with N. This was thought to be due to GA reducing N content shortly after application allowing for increased clover growth and N production from the nodules.

2.4 Effect of N

The commonly used approach to increase the DM production of perennial ryegrass white clover pastures is to apply N fertiliser (Ball & Field, 1982). N fertiliser initially typically increases protein content in pasture which, will then slowly decrease after 2 -3 weeks because enhanced pasture growth over this Period dilutes the luxury uptake and returns crude protein levels to normal (Ball & Field, 1982). In addition leaf area increases due to an increase in cell elongation, resulting in longer thinner leaves and an increased number of tillers because of an increased ability to intercept light. This occurs because leaves are able to produce more photosynthate (Woledge, 1988). Despite this increase in growth and tiller number after a while tiller numbers decline because of competition (Woledge, 1988). With N application dry matter percentage decreases owing to the greater water content of the larger leaf cells (White & Hodgson, 2000).

The rate of return from nitrogen can vary greatly depending on environmental (rain, temperature and soil moisture) and pasture (age and quality) conditions. On a perennial ryegrass seed crop in Canterbury New Zealand total plant uptake of late winter and spring applied N was greater (40 and 43% respectively) than N fertiliser applied in autumn (18%) in the years 1996 and 1997. This meant plant demand for N was higher in late winter and early spring than in autumn (Cookson, Rowarth, & Cameron, 2001). The reason for this could be because in New Zealand the stock have been wintered off onto brassica crops which leads to decreased urine N inputs, there is also a decreased level of N fixation by clover during this period because of low soil temperatures and an increased level of N leaching under the higher rainfall conditions. Whereas in autumn the demand for nitrogen may be lower because of the accumulative N inputs throughout the rest of the year including N fixation by legumes. A trial done in Southland, New Zealand found mean annual pasture responses to applied fertiliser N of a permanent ryegrass and white clover were 14.8, 12.9 and 9.1 kg DM/ kg N applied in the 100, 200 and 400 kg N treatments respectively. The authors concluded that greater responses were to be observed in spring compared with autumn in 3 out of the 4 years during the experiment (Monaghan, Paton, Smith, Drewry & Littlejohn, 2005). However as a rule of thumb, N fertiliser responses on intensively managed pastures are usually 5-10 kg DM/kg N applied (Ball, Molloy & Ross, 1978). N responses can be maximised by only applying fertiliser when conditions are suitable for responsive pasture growth, but in reality feed shortages usually occur when environmental conditions for pasture growth are poor. To improve the efficiency of the pasture response to N farmers can use the lowest rate of N fertiliser possible to provide the feed required, and by spelling the pasture (not grazing) for 4-6 weeks after N fertiliser is applied. This also reduces N losses (White & Hodgson, 2000).

2.5 GA and N

All previous studies using a GA and N treatment were conducted on perennial ryegrass white clover pastures and, in general, two weeks following the application of GA and nitrogen, the GA and N treated plots were found to show a similar response to that of GA applied by itself, with an increase in yield and stem elongation (Scott, 1959). In the same trial on day 41 a yield cut was made and it was found that GA plus N produced a similar yield as GA only (1333 vs. 1243 kg DM/ha respectively). The yield response remained similar between the two treatments up until day 207 post application. No interaction was found for pasture yield increase when the two treatments were applied together (Scott, 1959). In another trial the combination of GA and N outperformed GA treated pastures, though the differences were not large, it was thought that the increase in productivity was mainly due to increased productivity of clover from the application of N (Biddiscombe et al., 1962b).

Nutrient transfer required by either animals or fertiliser to gain optimal growth response from GA means N should not be limiting (Whitney, 1976). In a study done with two tropical grasses it was found that GA (62g GA/ha + N (68 – 180kg N/ha) gave an average yield response of 1260 kg DM/ha over the control in a Period of four years (Whitney, 1976). This study reveals that when GA is applied with N and N is not limiting GA application can provide sustainable growth responses from tropical grasses. This is yet to be seen with temperate grasses grown in a temperate environment.

An experiment done in New Zealand supported the above findings and concluded that when GA and nitrogen are applied together, this could remedy some of the negative side effects experienced by plants when subjected to a single application of GA. It was stated that gibberellins would be expected to cause a modest increase in herbage fibre content and to decrease herbage crude protein content and tiller density, while N fertiliser would be expected to do the opposite (Matthew et al., 2009). Data from both of the trials done by Matthew and his colleagues in 2009 have shown this to be true. Despite not having a combination treatment of GA and N in the first trial, the trial site was on an intensively grazed dairy pasture which is assumed not to be limited in N content. In this first trial done in early spring crude protein content and acid detergent fibre were found to be similar between control and GA treated plots (12.6 vs. 12.4% CP and 26.9 vs. 27.3% ADF for control and GA treated pastures respectively). In the second experiment a combination was used with two separate N fertiliser rates (40 and 80 kg N/ha). Tiller density was not affected by GA application and yield was found to be 290 and 390 kg DM/ha more than GA only pasture for GA plus N (40 kg N/ha) and GA plus N (80 kg N/ha; (Matthew et al., 2009)).

In a recent study done in New Zealand dry matter yields were found to be increased by 36 and 23% in winter and spring trials respectively over that of the control, but the next harvest was found to have no significant difference between treatments in regards to yield, and N treatments were found to be higher in early spring (Jiang, Carey, Roberts, & Kerse, 2010). This is further supported by a trial done recently in New Zealand where an interaction between GA and N was found for a winter derived pasture (a pasture grown in short days and given a single application of GA). The authors stated that the pasture response from GA application after 3 weeks of regrowth was better in pastures subjected to high N (69.6%) rather than low N (15.2%). However at week 4 of regrowth GA increased growth in pasture at both high and low N content (Parsons et al., 2013). This increase in yield with the GA and N treatment on a perennial ryegrass and white clover pasture further supports previous research that when applied together the response of GA and N is complementary and additive.

2.6 Conclusion

- GA is one of four plant growth regulators which can significantly alter the growth of plants.
- Bioactive gibberellins achieve this by breaking down DELLA proteins which act as growth suppressors, and work in conjunction with other growth regulators to promote out of season growth.
- GA can be applied to pasture and used to increase dry matter production to between 200-500 kg DM/ha.
- GA has a variable effect on plants depending on plant species, time of year and how many times GA is applied.
- N application causes a response of between 5 – 10 kg DM per kg N applied.
- When applied to pasture together, the response of GA and N is complementary and additive.
- There is limited research on the effect of GA on autumn pasture productivity.
- Research so far is inconclusive as to whether more than one application of GA is detrimental or not to pasture production.

Chapter 3
Materials and Methods

3.1 Experimental site

The trial was conducted in 2012 two separate experiments on the Lincoln University research dairy farm (LURDF) in Canterbury New Zealand (43o38'S, 172o27'E).. The soil type at the experiment is a Wakanui silt loam. The first experiment (autumn experiment) took place in autumn, beginning in the middle of March (15/3/12) and continued until the middle of May (16/5/12). The second experiment (spring experiment) commenced in spring in early august (2/8/12) and continued until early October (3/10/12). The experimental area was a part of the existing milking platform area of a dairy farm. Separate areas of pasture were used for the autumn and spring experiments. The areas were rotationally grazed with dairy cows to a low residual on a regular basis until each of the two experiments was conducted.

The pasture was a mixture of perennial ryegrass (*Lolium perenne*; cv Trojan, AR37 endophyte, heading date + 16 days) and white clover (*Trifolium repens*; cv Weka) sown at 18 and 4 kg seed/ha, respectively. Pasture was sown with a coulter drill following ploughing and cultivation on 15 April 2011. Soil tests taken at the site before the experiment started showed pH of 5.7, Olsen P of 20, K of 4 and sulphate sulphur of 28. A total of 400 kg/ha of 10% Potash super phosphate was applied at the end of the autumn experiment.

Climate data for the experimental area were sourced from the Broadfield Experimental Station located 1 km from the experimental site. The site was irrigated with a spray irrigator throughout the year. A total of 499 mm was applied as irrigation from October 2011 to October 2012. There were two applications in March 2012 (55 mm total for month). Rainfall and soil temperature are shown in figure 3.1 (NIWA, 2013).

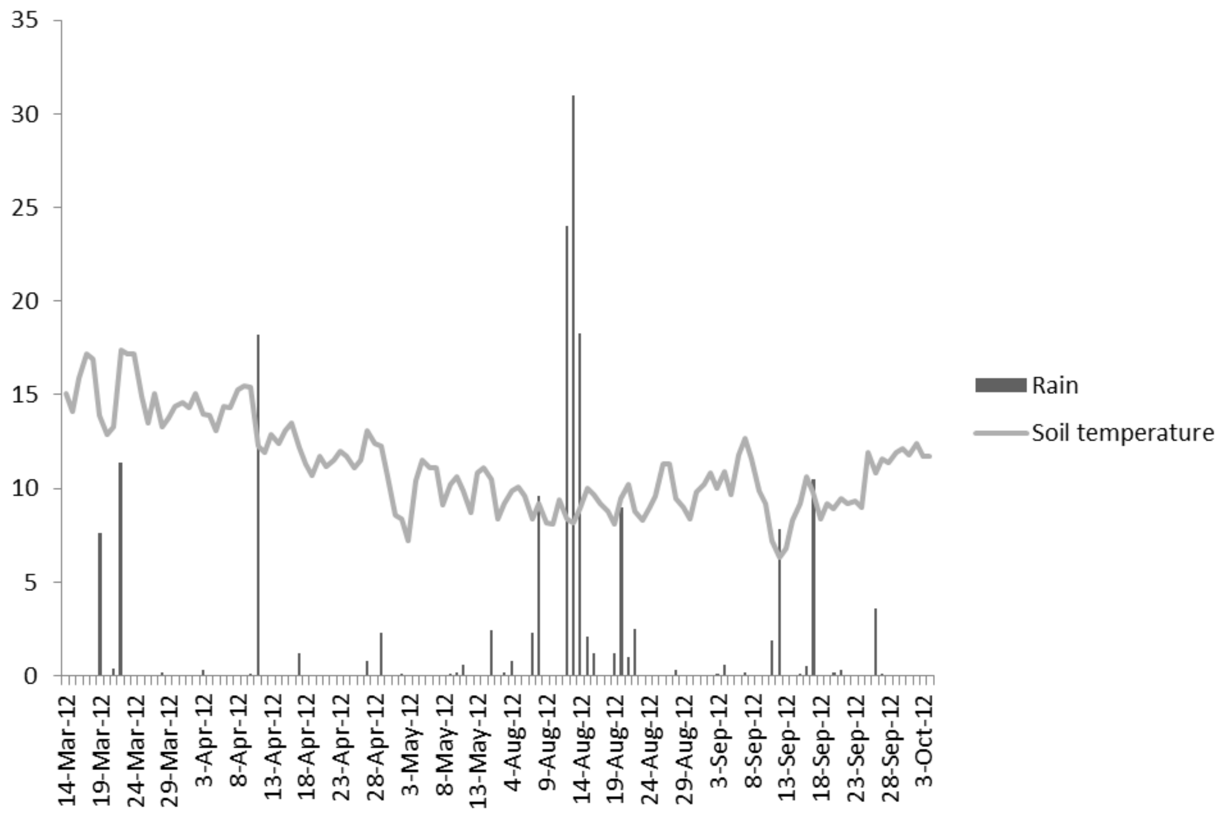

Figure 3.1: Rainfall and soil temperature for the experimental Period (NIWA, 2013).

3.2 Experimental design

Period 1

Both the spring and autumn experiments were a split plot design with four replicate blocks. The pasture were mown to a height of 4.5 cm with a rotary mower, and assigned to 4 main plot treatments on 15 March 2012: control (0 N and 0 GA), GA applied at 8g/ha, N applied at 40 kg N/ha and a combination of GA applied at 8g GA/ha and N applied at 40kg N/ha. GA was applied as ProGibb © SG (40% GA). A total of 1.6g of ProGibb was mixed with 16 L of water, the surfactant Contact was added (4 ml); this mixture was poured into a 16 L knapsack. The ProGibb foliar spray was applied with a double nozzle wand, with each nozzle producing 1.2 L water per minute. The person applying the GA walked at 5 km/hour for an even spread. Wand nozzles were held at a height of 20-30 cm above ground. Nitrogen was applied by hand spreading of granular urea (46% N).

Period 2 and long term response

After harvest after 28 days, each main plot was divided into four subplots (2 x 8 m), giving a total of 64 subplots over four replicates. Three of the four subplots were mown to 4.5 cm height with rotary mower and randomly assigned to three treatments: control (0 N and 0 GA);

GA applied at 8g/ha; N applied at 40 kg N/ha (Period 2). The fourth subplot was left unharvested and not treated with GA or N and thus measured the long term response (8 week harvest interval) to the initial applications of GA and N.

In the autumn experiment, Period 1 began on 15 March 2013, and Period 2 on 12 April 2012, with a final harvest on 16 May 2012. In the spring experiment, Period 1 began on 2 August 2012, and Period 2 on 30 August 2012, with final harvest on 3 October 2012. A yield cut was taken (20/8/12) to assess if there is a long term effect of autumn applied GA and N on pasture dry matter production following the winter Period.

3.3 Measurements

The same set of measurements were conducted in both the autumn and spring trials, except root mass which was very labour intensive so only measured in autumn.

3.3.1 DM yield

Mower yield

Pasture was harvested using a rotary mower after 28 (16 April 2012, Period 1) and 56 days (16 May 2012, Period 2). One strip was mown the length of the 8 m plot to a height of 4.5 cm (approximately grazing height). The bulk fresh weight was recorded and a sub sample dried in the oven at 60 ^{o}C oven for 48 hours.

Quadrat cut yield

Herbage in one 0.2 m^2 quadrat was cut with electrical hand shears to 1 cm height above ground level in each of the main plots and sub plots. Samples were collected after 28 (Period 1) and 56 (Period 2) days. Three samples were taken per main plot in Period 1 and one quadrat per sub plot in Period 2. Samples were dried at 60 ^{o}C oven for 48 hours, and weighed.

Rising plate meter

Compressed pasture height was measured using a rising plate meter. The Jenquip F150 electronic pasture meter was placed at 40 random locations in each main plot on day 28 (Period 1) and 20 locations in each sub plot on day 56 (Period 2).

3.3.2 Botanical composition

Snip samples were taken after 28 (Period 1) and 56 (Period 2) days. Electric hand shears were used to cut pasture to grazing height (4.5 cm). Four samples were cut at random per plot. A subsample was sorted into the dead, weed, vegetative or reproductive grass and clover components. Once separated, the samples were placed into the 60 ^{o}C oven for 48 hours. After this, dry weights were recorded. Dry weights were used to calculate the proportion of each species. These proportions were then calculated with the yield of the mower to get relative proportion in kg DM/ha.

Nutritive value

The 50 g sub sample of mixed pasture from the botanical composition sample was placed into airtight plastic bags and directly put into the freezer, before being freeze dried. Dried samples were ground through a 1 mm sieve (Retsch grinder) and the chemical composition (ADF, NDF, DMD%, OM%, CP%, N% WSC) estimated using near infrared spectrophometry (NIRS) (FOSS, NIRSystems 5000, Maryland, USA) as described by Bryant et al. (2012).

3.3.3 Sward structure

Standing sward height

Standing pasture height was measured after 28 (Period 1) and 56 (Period 2) days by placing a ruler at ground level (Piggot, 1989) at 20 random locations in each main plot (Period 1) and 10 locations in each sub plot (Period 2).

Leaf length, leaf width, pseudo stem length and true stem length

Sward structure was measured after 28 (Period 1) and 56 (Period 2) days. Ten tillers were selected at random from 2 cores (722 cm^3) taken from each main and sub plot. Leaf width (mm), leaf length of youngest fully emerged leaf (mm), pseudo stem length (mm), and true stem length (mm) were measured with a ruler and recorded. Pseudo stem was defined as the stem of the plant from the base where roots attach to the youngest fully emerging leaf. True stem was defined as the part of the pseudo stem at the base of the plant where the stem is the most fibrous.

Tiller density

Hand shears were used to cut one 0.01m^2 quadrat to ground level in each main plot and sub plot. The numbers of tillers per quadrat were counted. Cuts were made after 28 (Period 1) and 56 (Period 2) days. Tillers per m^2 were calculated.

3.4 Data analysis

Data was analysed using Genstat (v.13). DM production, DM content, botanical composition, sward surface height and nutritive value for the initial Period 1 were analysed by factorial analysis of variance (ANOVA) of a randomised complete block design. Treatments were GA (with and without) and N (with and without). Data in Period 2 were analysed by ANOVA of a split plot design with factorial combinations from Period 1 as the main plot factor and treatment in Period 2 (GA, N) as the subplot factor. In all cases, where there was more than one sample per plot, analysis was based on the mean value per main plot or sub plot. Long term response data was analysed separately using a general ANOVA for all variables.

Chapter 4- Results

4.1.1 Climate

In autumn a total of 46.1 mm of rain fell between the Period of 15 March until 16 May 2012. In Period 1 a total of 38.2 mm fell and 7.9 mm in Period 2. In spring Period 1 had 103.5 mm rainfall and Period 2 had 25.9 mm with a total of 129.4 mm (2 August til 3 October) in spring. Average soil temperature at 10 cm decreased from 14.6°C in Period 1 to 10.9°C Period 2 with an average of 12.6°C in autumn. In spring average soil temperature at 10 cm was 10.2°C increasing from 9.2°C in Period 1 to 11.1°C in Period 2 (Figure 3.1).

4.2 Autumn

4.2.1 Period 1

Mower DM yield was 31% higher (2152 vs. 1496 kg DM/ha) in N-fertilised compared with unfertilised pasture (Table 4.1). There was no significant effect at end of Period 1 of GA or interaction of GA x N on mower yield, quadrat cut yield, or SSH (RPM (Table 4.1)). N-fertilised pasture was 18% taller (10.5 vs. 8.6 cm) than unfertilised pasture (Table 4.1).

Table 4.1: DM yield (mower and quadrat cut) and sward height (RPM) of pasture treated with GA, N or a combination (GAN) in Period 1 in autumn. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period 1	**mower yield kg DM/ha**	**quadrat cut yield kg DM/ha**	**RPM cm**
O	1329	2553	8.5
GA	1662	2597	8.8
N	2076	2580	10.5
GAN	2227	3251	10.5
P value			
GA	0.09	0.07	1.71
N	**<.001**	0.08	**<.002**
GA x N	0.50	0.10	1.74
SED	**182.30**	**240.20**	0.48

N-fertilised pasture had 44% higher ryegrass yield (1562 vs. 880 kg DM/ha), 10% higher crude protein (21.0% vs. 18.9%) and 1% higher ME (12.4 vs. 12.2 MJ ME/kg DM) than unfertilised pasture (Table 4.2). There was no significant effect of GA or interaction of GA and N on ryegrass yield, clover yield, CP% or ME (Table 4.2).

Table 4.2: DM yield of total herbage, ryegrass, clover dead and weed, and DM%, CP% and ME pasture collected from lawn mower cut to 4.5 cm that was treated with GA, N or a combination (GAN) in first Period (28 days) in autumn. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period 1	**ryegrass**	**clover**	**dead**	**weed**	**DM content**	**CP**	**ME**
		kg DM/ha			**%**	**%**	**MJME/kg DM**
O	777	489	58	5	16.9	18.6	12.2
GA	982	602	79	0	16.5	19.1	12.2
N	1578	432	66	0	16.2	20.5	12.4
GAN	1546	606	74	2	15.9	21.5	12.3
P value							
GA	0.56	0.15	0.42	0.52	0.27	0.36	0.68
N	**0.001**	0.78	0.93	0.51	0.09	**0.03**	**0.004**
GA x N	0.43	0.75	0.71	0.19	0.87	0.73	0.96
SED	**204.00**	**129.60**	**23.55**	**3.34**	**0.52**	**1.16**	**0.06**

GA treated pasture had 12% longer pseudo stem (68.45 vs. 60.5 mm) than pasture not treated with GA (Table 4.3). GA application had no significant effect on SSH (ruler) or root mass (Table 4.3). N-fertilised pasture was 15% taller (SSH (ruler) 23.5 vs. 20.1 cm), had pseudo stems that were 11% longer (68.4 vs. 60.6 mm), and leaf length that was 12% longer (168.2 vs. 148.6 mm) than unfertilised pasture (Table 4.3). There was no significant effect of N on leaf width, true stem length or root mass (Table 4.3). There was a significant interaction between GA and N for pseudo stem and leaf length (Table 4.3). With no N applied, GA application had no significant effect on pseudo stem or leaf length. With N applied, GA increased pseudo stem length by about 22% and leaf length by about 18%. N fertilised pasture contained 23% more tillers (11,547 vs. 8,932 tillers/ m^2) than unfertilised pasture.

Table 4.3: Sward surface height (SSH, ruler), pseudo stem length, leaf length, leaf width, true stem length and root mass of pasture that was treated with GA, N or a combination (GAN) in Period 1 in autumn. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period 1	SSH cm	Pseudo stem mm	leaf length mm	leaf width mm	true stem mm	root mass g/corer	tiller density tiller/m^2
O	18.46	61.2a	153a	3.45	3.12	5.6	8038
GA	21.65	60a	144.3a	3.45	4.65	5.57	9825
N	24.29	59.8a	153.5a	3.65	3.35	5.94	11800
GAN	22.83	76.9b	182.8b	3.55	5.69	6.39	11294
P value							
GA	0.46	**<.001**	0.09	0.64	**0.02**	0.83	0.44
N	**0.01**	**<.001**	**0.002**	0.16	0.43	0.54	**0.01**
GA x N	0.07	**<.001**	**0.002**	0.64	0.61	0.80	0.18
SED	**1.59**	**2.94**	**8.61**	**0.15**	**1.13**	**1.29**	**1111.60**

4.2.2 Period 2

There was no effect of GA applied on quadrat cut DM yield at the end of Period 2 (Table 4.4). Mower yield was higher in GA treated (875 kg DM/ha) than untreated (528 kg DM/ha) but was similar to N-fertilised pasture (712 kg DM/ha; Table 4.4). SSH (RPM) was higher in GA (6.9 cm) treated than untreated (5.8 cm) pasture but was not different from N-fertilised (6.3 cm) pasture (LSD = 0.656; Table 4.4). Mower yield and SSH (RPM) were greater at end of Period 2 in pastures treated with N in Period 1 than pastures that were not treated with N (749 vs. 661 kg DM/ha; 6.6 vs. 6.0 cm). There were no significant interaction for mower yield; quadrat cut yield or SSH (RPM) of application of GA and N in Period 1 and 2.

Table 4.4: DM yield (mower cut and quadrat cut), and sward surface height (RPM) measured at the end of Period 2 in autumn of pasture that received factorial combinations of GA and N in Period 1 and then GA, N or nothing in Period 2. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N x P2 interaction.

Period 1	Period 2	Mower yield kg DM/ha	Quadrat cut yield kg DM/ha	RPM cm
O	O	536	2090	5.4
	GA	802	2465	6.6
	N	591	2355	5.9
GA	O	478	2144	5.6
	GA	861	2610	6.6
	N	699	2580	6.1
N	O	574	2450	6.2
	GA	993	2301	7.4
	N	837	2508	6.7
GAN	O	525	2630	6.0
	GA	844	2845	6.8
	N	720	2630	6.5
P value				
GA		0.38	0.08	0.41
N		**0.03**	0.12	**<0.001**
GA x N		0.07	0.55	0.09
P2		**<0.001**	0.24	**<0.001**
GA x P2		0.86	0.71	0.53
N x P2		0.63	0.35	0.91
GA x N x P2		0.39	0.68	0.86
SED		**93.7**	**283.40**	**0.32**

Clover DM yield was greater at end of Period 2 in pastures treated with GA (240.4 kg DM/ha) than N fertilised (80.1 kg DM/ha) and control pastures (89.0 kg DM/ha) (LSD = 43.16; Table 4.5). Ryegrass DM yield was greater in both GA (624 kg DM/ha) and N fertilised (623 kg DM/ha) than control pasture (423 kg DM/ha) (LSD = 84.4; Table 4.5). At the end of Period 2 GA treated pasture had a lower crude protein content (19.29 %) than N fertilised (21.56 %) and control (20.00 %) pasture (LSD = 1.063; Table 4.5). N fertilised pasture had more grass (609.6 kg DM/ha) and lower DM content (19.5 %) than unfertilised (504 kg DM/ha and 20.6%). There were no significant interactions for the yield components, CP% or ME between application of GA and N in Period 1 and 2.

Table 4.5: Ryegrass, clover and dead content, nutritive value, and dry matter content at the end of Period 2 in autumn of pasture that received factorial combinations of GA and N in Period 1 and then GA, N or nothing in Period 2. P2 = treatment in Period 2. P values from ANOVA for effects of P2, GA, N, GA x N and interactions between treatments in Period 1 and 2 are shown. SED = Standard error of difference for GA x N x P2 interaction.

Period	Period	Clover	Ryegrass	Dead	Weed	DM content	Crude Protein	ME
1	2		kg DM/ha			%	%	MJME/kg DM
O	O	105.8	410.0	20.1	-	21.5	19.7	12.6
	GA	249.4	545.0	5.9	1.2	20.2	18.9	12.5
	N	77.2	505.0	9.3	-	21.1	20.8	12.6
GA	O	95.0	366.0	17.4	-	21.3	20.5	12.5
	GA	277.0	574.0	9.9	-	19.6	19.8	12.5
	N	68.7	624.0	7.0	-	20.4	21.5	12.5
N	O	90.2	469.0	14.5	-	20.3	19.4	12.6
	GA	200.5	776.0	15.9	0.9	19.0	18.7	12.5
	N	104.2	723.0	9.4	0.7	19.5	22.3	12.7
GAN	O	64.8	449.0	10.9	0.4	19.9	20.4	12.5
	GA	234.7	600.0	9.4	-	18.9	19.8	12.5
	N	70.4	641.0	8.8	-	19.5	21.7	12.5
P value								
GA		0.87	0.39	0.63	0.10	0.47	0.14	0.30
N		0.31	**0.004**	0.98	0.56	**0.02**	0.70	0.64
GA x N		0.75	0.07	0.69	0.97	0.70	0.68	0.94
P2		**<.001**	**<.001**	0.32	0.27	0.07	**<.001**	0.38
GA x P2		0.40	0.55	0.98	0.08	1.00	0.58	0.36
N x P2		0.37	0.77	0.54	0.69	0.95	0.56	0.98
GA x N x P2		0.93	0.30	0.80	0.58	0.91	0.74	0.92
SED		**42.43**	**83.00**	**9.71**	**0.56**	**1.11**	**1.05**	**0.11**

SSH (ruler) at end of Period 2 was greater in pastures treated with GA (23.5 cm) than N fertilised (19.9 cm) or control (17.4 cm; LSD = 1.241). Pseudo stem and leaf length at the end of Period 2 were greater in pastures treated with GA (74.46 and 169.7 mm) than N fertilised (62.95 and 147.1 mm) or control (66.86 and 138.7 mm) pastures (pseudo stem LSD = 4.103 and leaf length LSD = 9.31). For pseudo stem, true stem and leaf length, there was also a significant interaction between the effects of GA application in Period 1 and Period 2 treatment. For N fertilised and GA treated pastures in Period 2, there was no effect of GA application in Period 1. For control pasture, GA treated pasture in Period 1 had greater pseudo stem (150.1 vs. 126.7 mm) and leaf length (74.5 vs. 59.2 mm) but lower tiller density (8275 vs. 11088 tillers/ m^2) than untreated pasture. True stem was greater in control pasture in

Period 2 (10.4 cm) than pasture treated with GA (8.8 cm) and N fertiliser (5.0 cm). For N treated pasture in Period 2, GA application in Period 1 had no effect on true stem length. In contrast, for GA and control treated pasture in Period 2, GA application in Period 1, resulted in increased true stem length, fertilised and GA treated pastures in Period 2, there was no effect of GA application in Period 1. Root mass was unaffected by GA or N application in Period 1 and Period 2 (Table 4.6).

Table 4.6: Sward sufarce height (ruler), pseudo stem, leaf length, leaf width, true stem length, root mass and tiller density at the end of Period 2 in autumn of pasture that received factorial combinations of GA and N in Period 1 and then GA, N or nothing in Period 2. P2 = treatment in Period 2. P values from ANOVA for effects of P2, GA, N, GA x N and interactions between treatments in Period 1 and 2 are shown. SED = Standard error of difference for GA x N x P2 interaction. Means within column are significantly different according to LSD test, follow significant ANOVA.

Period	Period	SSH	Pseudo stem length	leaf length	leaf width	true stem	root mass	Tiller density
1	2	cm	mm	mm	mm	mm	g/corer	tiller/m^2
O	O	16.4	54.3a	105.5a	3.0	9.1	2.2	10475
	GA	22.7	73.2de	168.8efg	3.5	6.6	2.4	8475
	N	18.8	61.4abc	130.1b	3.1	4.0	1.8	10550
GA	O	15.8	80.5e	168.4efg	3.7	16.1	2.4	8100
	GA	23.6	77e	160.9defg	3.5	10.5	2.1	5950
	N	19.8	60.1ab	155.7def	3.7	6.9	2.6	10500
N	O	18.1	64.1bc	147.6bcd	3.5	5.3	2.1	10575
	GA	24.6	73.7de	175.6g	3.4	5.6	2.1	13400
	N	20.6	67.6bcd	149cd	3.3	6.0	2.0	13050
GAN	O	19.5	68.5cd	133.3bc	3.8	11.0	2.5	7375
	GA	23.4	73.9de	173.3fg	3.7	12.7	1.9	8675
	N	20.6	62.8bc	153.6de	4.0	3.1	2.1	9050
P value								
GA		0.62	**0.01**	**0.003**	**<.001**	**<.001**	0.38	**<.001**
N		**0.002**	0.69	0.07	**0.003**	**0.03**	0.44	0.07
GA x N		0.72	**0.01**	**<.001**	1.00	0.41	0.75	0.11
P2		**<.001**	**<.001**	**<.001**	0.86	**<.001**	0.72	0.11
GA x P2		0.85	**<.001**	**0.01**	**0.01**	**0.001**	0.17	0.66
N x P2		0.32	0.30	0.80	0.28	**0.02**	0.79	0.06
GA x N x P2		0.23	**0.05**	**<.001**	0.10	0.05	0.41	0.68
SED		**1.22**	**4.18**	**9.48**	**0.10**	**1.86**	**0.38**	**1754.60**

4.2.3 Long term response

N-fertilised pasture in the first Period had 20% greater mower DM yield ((2610 vs. 2097 kg DM/ha, P = 0.05) Table 4.7), 29% greater ryegrass yield (2109 vs. 1496 kg DM/ha) and 41% lower clover yield (239 vs. 402 kg DM/ha) than unfertilised pasture when measured in pastures that were not harvested until 62 days (Table 4.8). GA application had no significant effect on mower DM yield when measured in pastures that were not harvested until 62 days.

Table 4.7: DM yield (mower and quadrat cut) and sward height (RPM) of pasture 62 days post treatment with GA, N or a combination (GAN) in Period 1 in autumn. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period 1	Period 2	Mower yield kg DM/ha	Quadrat cut yield kg DM/ha	RPM cm
O	-	2148	3485	9.8
GA	-	2045	3420	9.9
N	-	2598	4865	11.4
GAN	-	2621	4291	10.6
P value				
GA		0.87	0.19	0.47
N		**0.05**	**<0.001**	**0.03**
GA x N		0.79	0.29	0.40
SED		**325.40**	**318.20**	**0.63**

Table 4.8: DM yield of total herbage, ryegrass, clover dead and weed, and DM%, CP% and ME pasture after 62 days of herbage that was treated with GA, N or a combination (GAN) in Period 1 in autumn. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period 1	Period 2	Clover	Ryegrass	Dead	Weed	Dry matter content	Crude Protein (%)	ME MJME/kg DM
		kg DM/ha						
O	-	475	1484	189	-	21.6	16.1	12.3
GA	-	329	1508	208	-	21.7	15.0	12.2
N	-	234	2122	242	-	21.0	15.5	12.4
GAN	-	244	2096	281	-	21.7	16.6	12.4
P value								
GA		0.37	1.00	0.42	**-**	0.49	0.97	0.13
N		**0.05**	**0.01**	0.10	**-**	0.61	0.50	**0.02**
GA x N		0.30	0.89	0.77	**-**	0.62	0.14	0.82
SED		**101.30**	**262.80**	**48.10**	**-**	**0.80**	**0.98**	**0.09**

Table 4.9: Sward surface height (SSH, ruler), pseudo stem length, leaf length, leaf width, true stem length, root mass and tiller density) of pasture 62 days post treatment with GA, N or a combination (GAN) in Period 1 in autumn. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period	Period	SSH	Pseudo stem length	leaf length	leaf width	true stem	root mass	Tiller density
1	**2**	**cm**	**mm**	**mm**	**mm**	**mm**	**g/corer**	**tiller/m²**
O	-	29.7	86	212.3ab	9.7	7.7a	2.4	11175b
GA	-	29.1	89.4	196.9a	3.2	9a	2.7	6425a
N	-	31.9	110.6	220.9b	3.9	11.3a	2.6	9825ab
GAN	-	32.55	123.9	275.4c	4.3	25.5b	2.4	12850b
P value								
GA		0.98	0.08	**0.02**	0.30	**0.001**	0.88	0.52
N		**0.04**	**<.001**	**<.001**	0.43	**<.001**	0.95	0.08
GA x N		0.61	0.29	**<.001**	0.25	**0.01**	0.38	**0.02**
SED		**1.69**	**6.65**	**11.32**	**4.18**	**3.41**	**0.37**	**1837.50**

GA treated pasture had 8% longer leaves (266.2 vs. 216.6 mm) and 11% longer true stem (17.3 vs. 9.5 mm) than pasture not treated with GA in Period 1. N-fertilised pasture in the first Period had 9% greater SSH (ruler) (32.2 vs. 29.4 cm, P = 0.04), 25% longer pseudo stem (117.3 vs. 87.7 mm), 18% longer leaves (248.2 vs. 204.6 mm) and 55% longer true stem (18.4 vs. 8.4 mm) than unfertilised pasture when measured in pastures that were not harvested until 62 days. When GA was applied alone tiller density was lower than combination (GAN), N fertilised (N) and control (O) pasture. A combination of GA and N resulted in longer leaf and true stem length than GA alone, N alone or control pasture (Table 4.9).

4.3 Spring

4.3.1 Period 1

After 28 days growth pasture was 34% higher (1009 vs. 666 kg DM/ha) for mower yield, 40% taller (RPM; 10.5 vs. 6.3cm) and had 13% higher quadrat cut yield (1902 vs. 1653 kg DM/ha) for GA treated pasture than pasture not treated with GA (Table 4.10). N-fertilised pasture yielded 28% higher (973 vs. 702 kg DM/ha, mower cut) and 21% higher (1984 vs. 1572 kg DM/ha, quadrat cut), and was 15% taller (6.2vs. 7.27 cm) than pasture without N (Table 4.10). There was no interaction between GA and N after 28 days for mower yield; quadrat cut yield or sward height (RPM).

Table 4.10: DM yield (mower and quadrat cut) and sward height (RPM) of pasture treated with GA, N or a combination (GAN) in Period 1 (28 days) in spring. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N.

Period 1	mower yield kg DM/ha	quadrat cut yield kg DM/ha	RPM cm
O	516	1394	5.6
GA	888	1749	6.7
N	816	1912	6.9
GAN	1130	2055	7.6
P value			
GA	**0.006**	**0.02**	**<.001**
N	**0.02**	**0.001**	**<.001**
GA x N	0.76	0.27	1.20
SED	**135.80**	292.10	0.22

After 28 days GA treated pasture contained 33% more ryegrass (730 vs. 491 kg DM/ha), 53% more clover (168 vs. 79 kg DM/ha, P = 0.05) than pasture not treated with GA (Table 4.11). N-fertilised pasture had 35% higher ryegrass yield (738 vs. 483 kg DM/ha) and had 14% higher protein content (24.6 vs. 21.2%) than pasture unfertilised pasture (Table 4.11). There was no significant effect of interaction of GA and N ryegrass yield, clover yield, CP% or ME (Table 4.11).

Table 4.11: DM yield of total herbage, ryegrass, clover dead and weed, and DM%, CP% and ME pasture collected from lawn mower cut to 4 cm that was treated with GA, N or a combination (GAN) in Period 1 in spring. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period 1	ryegrass	clover	dead	weed	DM content	CP	ME
		kg DM/ha			%	%	MJME/kg DM
O	355	84	76	1.0	22.1	22.5	12.5
GA	611	158	113	5.8	19.8	19.8	12.5
N	627	74	116	0.0	20.1	24.9	12.5
GAN	849	178	94	8.3	18.1	24.2	12.5
P value							
GA	**0.03**	**0.05**	0.70	0.25	0.072	0.16	0.40
N	**0.03**	0.91	0.60	0.89	0.11	**0.01**	0.73
GA x N	0.86	0.72	0.17	0.76	0.83	0.38	0.98
SED	**134.50**	**57.60**	**27.73**	**7.56**	**1.49**	**1.63**	**0.06**

On day 28 GA treated pasture was 24% taller (SSH ruler; 20.0 vs. 15.2 cm), had 25% longer pseudo stem (73.1 vs. 55.1 mm) and 28% longer leaves (162.1 vs. 116.1 mm) than pasture not treated with GA (Table 4.12). N-fertilised pasture was 15% taller (SSH (ruler, 19.1 vs. 16.2 cm) and contained 44% more tillers (15,815 vs. 8,875 tillers/m^2) than unfertilised pasture (Table 4.12). There were no interactions found between GA and N after 28 days in Period 1.

Table 4.12: Sward surface height (ruler, SSH) pseudo stem length, leaf length, leaf width, true stem length and tiller density of pasture that was treated with GA, N or a combination (GAN) in first Period (28 days) in spring. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period 1	SSH - ruler cm	Pseudo stem mm	leaf length mm	leaf width mm	true stem mm	tiller density tiller/m^2
O	13.3	48.5	106.2	3.8	9.7	9050
GA	19.1	74.7	169.1	3.9	18.5	8700
N	17.0	61.6	125.9	3.8	9.6	17080
GAN	21.0	71.3	155.0	3.6	13.1	14550
P value						
GA	**<.001**	**0.03**	**0.002**	0.93	0.09	0.34
N	**<.001**	0.49	0.79	0.42	0.43	**<.001**
GA x N	0.13	0.25	0.14	0.42	0.45	0.46
SED	**0.78**	**9.61**	**14.52**	**0.25**	**4.73**	**1998.80**

4.3.2 Period 2

4.13: DM yield (mower and quadrat cut) and sward height (RPM) measured at the end of Period 2 in spring of pasture that received factorial combinations of GA and N in Period 1 and then GA, N or nothing in Period 2. P2 = treatment in Period 2. P values from ANOVA for effects of P2, GA, N, GA x N and interactions between treatments in Period 1 and Period 2 are shown. SED = Standard error of difference for GA x N x P2 interaction.

Period 1	Period 2	Mower yield kg DM/ha	Quadrat cut yield kg DM/ha	RPM cm
O	O	970	2548	6.7
	GA	1090	2194	6.9
	N	1310	2735	7.5
GA	O	829	1759	5.7
	GA	1298	2102	6.4
	N	1167	2385	7.4
N	O	1340	2350	7.3
	GA	1645	2535	8.2
	N	1678	2888	8.6
GAN	O	1147	2252	7.0
	GA	1292	2661	7.0
	N	1385	2886	8.4
P value				
GA		**0.01**	0.10	**0.001**
N		**<.001**	**0.02**	**<.001**
GA x N		**0.03**	0.09	0.95
P2		**<.001**	**0.01**	**<.001**
GA x P2		0.57	0.30	0.17
N x P2		0.87	0.59	0.97
GA x N x P2		0.16	0.71	0.20
SED		**137.50**	**292.70**	**0.38**

Mower DM yield measured at end of Period 2 was greater in pasture treated with GA (1331 kg DM/ha) and N (1385 kg DM/ha) than control pasture (1072 kg DM/ha; LSD = 139.9). Quadrat cut yield and SSH (RPM) were higher in pasture that was N-fertilised (2724 kg DM/ha, 8.0 cm) than pasture treated with GA (2373 kg DM/ha, 7.1 cm) and nothing (2227 kg DM/ha, 6.7 cm) in Period 2 (Table 4.13). At the end of Period 2 pasture treated with GA in Period 1 had lower mower DM yield and SSH (RPM) than pasture not treated with GA (988 vs. 1155 kg DM/ha, 6.4 vs. 7.0 cm). Mower DM yield (1244 vs. 900 kg DM/ha), Quadrat cut DM yield (301 vs. 2154 kg DM/ha) and SSH (RPM; 7.2 vs. 6.6 cm) measured at the end of Period 2 were greater in pastures fertilised with N in Period 1 than unfertilised with N in

Period 1 (Table 4.13). Mower DM yield was greater at the end of Period 2 in pastures that had been treated with N in Period 1 but unaffected by GA application in Period 1 (Table 4.13). There were no significant interaction for quadrat cut yield, tiller density or SSH (RPM) of application of GA and N in Period 1 and 2. Overall mower DM yield over both Period 1 and 2 (mower yield from Period 1 treatments added to the mower yield results from Period 2 treatments) was highest in GANGA pasture in autumn (3071 kg DM/ha) and GANN treated pasture in spring (2515 kg DM/ha; Table 5.2).

4.14: Botanical composition and nutritive value measured at the end of Period 2 of pasture that received factorial combinations of GA and N in Period 1 and then GA, N or nothing in Period 2. P2 = treatment in Period 2. P values from ANOVA for effects of P2, GA, N, GA x N and interactions between treatments in Period 1 and 2 are shown. SED = Standard error of difference for GA x N x P2 interaction.

Period	Period	Clover	Ryegrass	Dead	Weed	DM content	Crude Protein	ME
1	2		kg DM/ha			%	%	MJME/kg DM
O	O	161.0	789.0	8.3	12.2	20.5	19.8	12.4
	GA	386.0	652.0	29.4	22.9	19.8	18.8	12.3
	N	308.0	965.0	31.5	5.5	19.1	20.6	12.5
GA	O	294.0	474.0	43.9	17.4	20.6	19.5	12.4
	GA	437.0	796.0	51.7	13.0	18.6	18.9	12.2
	N	182.0	962.0	19.9	3.3	18.9	21.5	12.4
N	O	274.0	1038.0	23.1	4.5	19.0	19.9	12.4
	GA	371.0	1224.0	44.8	4.9	20.0	18.0	12.4
	N	383.0	1267.0	27.9	0.3	18.9	19.9	12.4
GAN	O	237.0	895.0	14.9	0.1	19.5	18.5	12.3
	GA	202.0	992.0	95.8	1.4	19.6	19.2	12.4
	N	154.0	1207.0	23.6	0.4	19.5	20.7	12.5
P value								
GA		0.11	0.07	0.19	0.50	0.70	0.63	0.36
N		0.53	**<.001**	0.48	**0.01**	0.49	0.32	0.44
GA x N		**0.04**	0.42	0.90	0.97	0.17	0.99	0.39
P2		0.06	**<.001**	**0.03**	0.16	**0.04**	**0.005**	0.10
GA x P2		0.07	0.25	0.25	0.69	0.16	0.24	0.63
N x P2		0.19	0.70	0.34	0.44	**0.01**	0.89	0.54
GA x N x P2		0.82	0.12	0.37	0.64	0.93	0.59	0.50
SED		**93.70**	**130.50**	**26.00**	**8.69**	**0.61**	**1.11**	**0.10**

Ryegrass DM yield was greater at end of Period 2 in pastures treated with GA (916 kg DM/ha), and N (1100 kg DM/ha) in Period 2 than control pastures (799 kg DM/ha; LSD =

132.7) (Table 4.14). CP% was greater at end of Period 2 in pastures treated with N(20.7%), and nothing (19.4%) in Period 2 than pastures treated with GA (18.7%; LSD = 1.15) (Table 4.14). Ryegrass yield was greater at the end of Period 2 in pastures that had been treated with N in Period 1 but unaffected by GA application in Period 1 (Table 4.14). There were no significant interactions for mower yield, CP% or ME between application of GA and N in Period 1 and 2.

SSH (ruler) at end of Period 2 was greater in pastures treated with GA (23.4 cm) than N fertilised (19.9 cm) or control (17.4 cm; LSD = 1.14). At the end of Period 2 pseudo stem was greater in pastures treated with GA (53 mm) and N (54 mm) than pastures treated with nothing (49 mm; LSD = 3.5). Leaf length at the end of Period 2 was greater in pastures treated with GA (146 mm) than N fertilised (136 mm) or control pastures (130 mm; LSD = 6.31). For pseudo stem and leaf width, there was also a significant interaction between the effects of GA application in Period 1 and Period 2 treatment. For N fertilised and GA treated pastures in Period 2, there was no effect of GA application in Period 1. For control pastures, GA treated pasture in Period 1 had greater pseudo stem (150.1 vs. 126.7 mm) and leaf length (74.5 vs. 59.2 mm) than untreated pasture. True stem was greater in control pastures in Period 2 (10.4 mm) than pastures treated with GA (8.8 mm) and N fertiliser (5.0 mm; LSD = 1.74). For N treated pasture in Period 2, GA application in Period 1 had no effect on true stem length. In contrast, for GA and control treated pasture in Period 2, GA application in Period 1, resulted in increased true stem length, fertilised and GA treated pastures in Period 2, there was no effect of GA application in Period 1 (Table 4.15).

4.15: Sward surface height (ruler), pseudo stem length, leaf length, leaf width, true stem length and tiller density of pasture measured at the end of Period 2 that received factorial combinations of GA and N in Period 1 and then GA, N or nothing in Period 2. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N x P2 interaction. Means within column are significantly different according to LSD test, follow significant ANOVA.

Period	Period	SSH	Pseudo stem length	leaf length	leaf width	true stem	Tiller density
1	2	cm	mm	mm	mm	mm	tiller/m^2
O	O	17.0	46.5ab	124.3	2.6	6.87ab	10210
	GA	19.3	40.8a	123.7	2.3	8.16abc	7875
	N	20.0	48b	117.8	2.4	8.28abc	9850
GA	O	15.8	51.1bcd	134.6	2.5	9.82bcd	7850
	GA	19.1	63.7f	162.2	2.9	15.36f	10800
	N	20.4	49.6bc	136.8	3.4	6.01a	11450
N	O	17.6	49bc	135.0	3.0	6.97ab	11150
	GA	21.2	55.2cde	154.3	2.5	11.22cde	9475
	N	21.5	57.9def	152.7	2.5	5.6a	10850
GAN	O	18.0	47.5ab	124.1	2.5	7.1ab	10200
	GA	22.1	52.2bcd	143.2	2.6	12.77def	10750
	N	22.6	61.1ef	137.2	2.8	13.4ef	10125
P value							
GA		0.61	**0.002**	0.05	0.06	**<.001**	0.76
N		**<.001**	**0.01**	**0.003**	0.84	0.55	0.44
GA x N		0.21	**<.001**	**<.001**	**0.05**	0.71	0.66
P2		**<.001**	**0.01**	**<.001**	0.50	**<.001**	0.75
GA x P2		0.62	**0.04**	0.06	**0.03**	0.27	0.30
N x P2		0.68	**0.004**	**0.02**	0.42	0.12	0.75
GA x N x P2		0.92	**<.001**	0.09	0.84	**<.001**	0.71
SED		**1.12**	**3.60**	**6.42**	**0.33**	**1.77**	**2367.70**

4.3.3 Long term response

N-fertilised pasture in Period 1 had 24% greater mower DM yield (3119 vs. 2358 kg DM/ha), 23% greater quadrat DM yield (4623 vs. 3556 kg DM/ha), and 21% taller (RPM) than unfertilised pasture when measured in pastures that were not harvested until 62 days (Table 4.16).

4.16: DM yield (mower cut and quadrat cut) and sward height (RPM) of pasture 62 days after treatment with GA, N or a combination (GAN) in Period 1 in spring. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period 1	Period 2	Quadrat cut yield kg DM/ha	Mower yield kg DM/ha	RPM cm
O	-	3645	2169	8.6
GA	-	3466	2547	9.8
N	-	4400	3015	11.7
GAN	-	4846	3223	11.5
P value				
GA		0.68	0.129	0.42
N		**0.01**	**0.002**	**0.002**
GA x N		0.35	0.638	0.23
SED		**443.20**	**247.9**	**0.80**

N-fertilised pasture had 30% greater ryegrass yield (2100 vs. 1473 kg DM/ha) than unfertilised pasture when measured in pastures that were not harvested until 62 days (Table 4.17). GA application had no significant effect on DM yield (mower or quadrat), RPM, botanical composition or nutritive value when measured in pastures that were not harvested until 62 days (Table 4.16 and 4.17).

4.17: Ryegrass, clover, dead and weed, and DM%, CP% and ME of pasture after 62 days of herbage that was treated with GA, N or a combination (GAN) in Period 1 in spring. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period	Period	Clover	Ryegrass	Dead	Weed	Dry matter	Crude Protein	ME
1	2		kg DM/ha			%	%	MJME/kg DM
O	-	662	1416	90a	0.4	21.0	18.4	12.5
GA	-	923	1530	81a	12.4	20.5	17.8	12.4
N	-	961	1930	80a	42.7	20.9	17.6	12.5
GAN	-	726	2269	193b	34.6	20.4	17.7	12.4
P value								
GA		0.92	0.11	0.07	0.91	0.09	0.66	0.19
N		0.70	**<.001**	0.07	0.10	0.68	0.42	0.99
GA x N		0.08	0.40	0.04	0.59	0.95	0.55	0.74
SED		**178.30**	**179.90**	**35.70**	**25.15**	**0.42**	**0.79**	**0.10**

After 62 days GA treated pasture was 10% taller (SSH – ruler; 27.7 vs. 24.8 cm) than pasture not treated with GA (Table 4.18). N-fertilised pasture had 24% longer pseudo stem (72.1 vs. 55.2 mm), 20% longer leaf (175.7 vs. 145.5 mm), 9% wider leaf (2.9 vs. 2.7 mm) and contained 30% more tillers (10800 vs. 7513 tillers/m^2) than unfertilised pasture (Table 4.18).

After 62 days pasture fertilised with N increased pseudo stem length and leaf length with and without GA. GA had little effect on pseudo stem length and leaf length on day 62 post treatment (Table 4.18).

4.18: Sward surface height (SSH, ruler), pseudo stem length, leaf length, leaf width, true stem length and tiller density of pasture 62 days post treatment with GA, N or a combination (GAN) in Period 1 in spring. P values from ANOVA for effects of GA, N and GA x N interaction are shown. SED = Standard error of difference for GA x N interaction.

Period	Period	SSH	Pseudo stem length	leaf length	leaf width	true stem	Tiller density
1	**2**	**(cm)**	**mm**	**mm**	**mm**	**mm**	**tiller/m^2**
O	-	22.45	54.4a	132a	2.67	12.2	7425
GA	-	26.62	55.9a	150.9b	2.64	13.1	7600
N	-	27.1	78c	193.1c	2.89	16.6	11025
GAN	-	28.77	66.2b	158.3b	2.93	13.1	10575
P value							
GA		**0.04**	0.08	0.19	0.96	0.32	0.92
N		**0.02**	**<.001**	**<.001**	**0.004**	0.09	**0.04**
GA x N		0.33	**0.03**	**<.001**	0.69	0.09	0.83
SED		**1.72**	**4.17**	**8.55**	**0.12**	**1.80**	**1964.80**

Chapter 5
General discussion

5.1 Introduction

The aim of this thesis was to quantify the effects of single and repeated application of GA and N fertiliser in autumn and spring on DM yield, pasture composition and morphology of a perennial ryegrass-white clover pastures in autumn and spring. This objective was considered by applying factorial combinations of GA and N in autumn and spring, completing an initial harvest after 28 days, before applying subsequent applications of GA, N or nothing. This approach thus, was able to consider the carryover effects of GA and N application.

5.1.1 DM response to initial GA

The initial application of GA in Period 1 increased DM yield when measured after 28 days with mower cuts by 242 kg DM/ha (12%) in autumn and 343 kg DM/ha (34%) in spring (P = <0.001). Previous research has reported a wide range of DM yield increases to GA application ranging from 50 to 980 kg DM/ha for temperate grasses (Wittwer & Bukovac 1957; Morgan & Mees 1958; Finn & Nielsen 1959; Scott 1959; Blacklow & McGuire 1971; Matthew *et al*. 2009; McGrath & Murphy 1976; Parsons et al., 2013). These studies typically show smaller responses in autumn than spring. This is thought to be due to use of younger pasture in these trials which have a limited amount of available root reserves, plant phenology and associated day length and temperature. For example, Parsons et al., (2013) found that GA treated plants derived from winter gave a much larger response than summer derived plants, mainly due to the seasonal changes in plant development having a major effect in the field on the capacity for the plants to respond to exogenous GA application. The results found in the current study confirm these findings because a larger increase in DM yield was found in spring compared to autumn**,** indicating that plant phenology is very important for a GA response.

The results from the current study also showed increased DM yield after N fertiliser application. The increases found in Period 1 were 656 kg DM/ha (31%) in autumn and 271 kg DM/ha (28%) in spring. These corresponded in high response efficiencies of 18.6 kg DM/kg N and 22.5 kg DM/kg N + GA in autumn, and normal range response efficiencies 7.5 kg DM/kg N and 15.4 kg DM/kg N + GA in spring (Table 5.1). This data contradicted the results found by Monaghan et al., (2005) who found that greater responses were to be observed in spring compared with autumn in 3 out of the 4 years during the experiment. The spring response efficiency is similar in the current experiment to that found in their experiment (16.0 kg DM/ kg N applied with an application rate of 100 kg N/ha). However response efficiency in autumn in the current experiment was higher than the response efficiency found in their experiment (13.6 kg DM/ kg N). The reason for this high N response efficiency in autumn is unknown, however we can theorise that a high rainfall environment can lead to N being lost from the soil, which means a lack of N in the system in autumn is likely and may have caused the high N response efficiency of the pasture after 28 days. It is noteworthy that the DM yield response to N application was larger than the DM yield response to GA application. The result for autumn is consistent with previous work conducted at the same site (Van Rossum *et al*. 2013). This showed that the response was 1583 and 1851 kg DM/ha respectively for N and N + GA application in autumn. The larger response in the earlier trial may reflect that GA was applied at the higher rate of 24g GA/ha.

Previous studies have noted that the relative responses to GA are greater at higher N fertility (Ball *et al*. 2012).This was confirmed in the current study, where GA applied with N achieved the maximum DM yield in both autumn and spring. This was also found in the study of van Rossum et al., (2013). However, of note is that there was no interaction between the effects of GA and N on DM yield. This indicates that the effects of GA and N in both autumn and spring were additive when applied together in a single application. This is the same as previous studies (Matthew *et al.,* 2009; Parsons *et al.,* 2013). Matthew et al., (2009) found that GA + 40 kg N/ha produced the second highest DM yield of 1620 kg DM/ha (lawn mower cut), and GA + 80 kg N/ha produced the highest DM of 1720 kg DM/ha. Similarly Parsons et al., (2013) found that winter derived plants treated with GA in a high N environment achieved the greatest DM yield. When GA is applied in conjunction with N regardless of the N environment (high or low), there was increased growth with no decline of N content within the plant. But they did make a note that growth of plants in an N saturated environment can reach a maximum growth rate due to the inability of plant tissue taking up any more N (Parsons et al., (2013).

5.1: Nitrogen response efficiency in autumn and spring with a single treated of N or N + GA. Response is mower yield acheived by treatment minus control yield.

Time	Treatment	N applied kg/ha	Response kg DM/ha	Efficiency kg DM/kg N
autumn				
day 28	N	40	744	18.6
	N + GA	40	898	22.5
spring				
day 28	N	40	300	7.5
	N + GA	40	614	15.4

5.1.2 DM response to repeat GA application

Previous studies have demonstrated reductions in DM yield at successive harvests following GA application (Morgan & Mees 1958; Finn & Nielsen 1959), which is often associated with reduced tiller densities. In this study, the application of GA in Period 2 increased DM yield when measured after 28 days with mower cuts by 347 kg DM/ha (40%) above the control in autumn and 259 kg DM/ha (19%) above the control in spring. Of note, is that the increase in DM yield was lower than in Period 1 for autumn but higher in Period 1 for spring. The difference in response between Period 1 and 2 may reflect changes in the climate constraints on plants as well as altered phenology. In autumn, Period 2 was colder (10.9^{o}C) so growth may have been more limited. While in spring, Period 2 was both warmer (11.1^{o}C), and plants approached flowering.

Measurements of yield at the end of Period 2 allowed carryover effects of GA application to be determined. In both spring and autumn, no interactions were found between GA and N application between Period 1 and Period 2. Thus, there was no evidence of yield depression with repeat application of GA as found in previous literature (Matthew et al., 2009; Parsons et al., 2013), and no carryover effects from Period 1 to 2 of application. The difference between this study and previous studies is not clear; however, it may reflect the different rates of GA application used in this trial (8g GA/ha).

5.1.3 GA and root growth

Earlier work used higher rates of GA application (24 - 105g GA/ha) than the current study and this has typically been associated with increased root growth (Finn & Nielsen, 1959), but in some cases GA application has caused short term root depression (Parsons et al., 2013). Parsons et al., (2013) discovered that GA treatment caused a reduction in winter derived plant root mass, but root mass recovered so much so that by week 4 of the experiment root mass in the low N, GA treated plants was 81% greater than in the low N control plants. In contrast, in the current study GA did not have an effect on root mass. So it is proposed that the lack of response by the roots of pasture in the autumn growth period was due to an abundance of N in the soil, with a little bit of help from the large amount of white clover fixing N into the soil solution as well.

Another theory is that GA depletes/utilises all available plant nutrients for shoot development, and there is a secondary compensation of roots by increasing mass to access a larger amount of nutrients from the soil. This means that autumn applied GA (repeated application or not) may not have had a detrimental effect on root production, but it is thought that below ground biomass could have further increased above ground plant biomass because of the roots being able to access more nutrients underground (Finn & Nielsen, 1959). The lack of response of root production indicates that the shoots may have out-competed the roots for the applied GA.

5.1.4 Increased yield and farmers options

The combined yield from mower cuts over Period 1 and 2 ranged from 1865 kg DM/ha (O P1 + O P2) to 3071 kg/DM ha (GAN P1 + GA P2) in autumn (56 days) and from 1486 kg DM/ha (O P1 + O P2) to 2515 kg DM/ha (GAN P1 + N P2) in spring (62 days; Table 5.2). The lowest value was achieved in spring (O P1 + O P2) and highest value in autumn (GAN P1 + GA P2) treatment (note this data was not analysed via statistical analysis). The practical importance of this is that integrating GA into an intensive farmland system with an abundance of N and other nutrients, can significantly improve DM production at times of the year when DM production is generally low. Increasing DM yield at these critical time periods (late autumn and early spring) has been found to increase live weight gain in weaner sheep and pregnant ewes (Biddiscombe, 1962).

Increased DM yield is able to give a farmer options in terms of retaining livestock longer on farm to maximise days in milk and body condition before the winter period (in the South island of New Zealand the general practice is to winter livestock on a forage crop such as kale or turnips; Holmes et al., 2007; Morgan & Mees, 1958). Having adequate amount of pasture will enable farmers to keep young stock on farm for longer, even when weaned, and to grow out young stock at a faster rate, having enough feed for both livestock classes (mixed age cows/ sheep and calves/ lambs). This will also give cows the potential to reach peak milk production faster and to reach target body condition for mating (Holmes et al., 2007). As a farmer having more options is far better than the alternative and GA is a good way to improve pasture production in the shoulders of the season to give the farmer those options. My advice would be to use GA within a grazing round with N, and then applying GA by itself the following round in both the autumn and spring. The rest of the season maintaining the applications of other nutrients such as N is essential to maintaining good soil and plant fertility to maximise growth of pasture.

5.2: Total mower DM yield achieved in autumn and spring under various treatment combinations from Period 1 and Period 2.

		Total yield (kg DM/ha)	
Period 1	**Period 2**	**autumn**	**spring**
O	**O**	1865	1486
O	**GA**	2131	1606
O	**N**	1920	1826
GA	**O**	2140	1717
GA	**GA**	2523	2186
GA	**N**	2361	2055
N	**O**	2650	2156
N	**GA**	3069	2461
N	**N**	2913	2494
GAN	**O**	2752	2277
GAN	**GA**	3071	2422
GAN	**N**	2947	2515

5.1.5 DM yield response in long term

The DM yield response to GA application on day 62 was minimal (long term response) in both autumn and spring. This confirms findings by previous authors that GA accelerates growth for a “short” (less than 4 weeks) time Period when grass growth is slow (mainly in spring and autumn) (Davies, 2010; Morgan & Mees, 1958; Scott, 1959; van Rossum et al., 2013). Morgan & Mees (1958) saw that GA could be used as a tool to extend the growing season and early and late ‘bite’ in autumn and spring, but that over time N fertiliser was still the best option to improve DM production. In support of findings by Morgan & Mees, 1958, Scott 1959 stated that marked and rapid growth responses (less than 41 days) could be obtained from GA application, and that a similar growth response could be achieved with N fertiliser (particularly in the cooler temperatures of winter) over a longer time period. The growth response had disappeared 105 days post application (Scott, 1959). The reason is due to the fact that GA mobilises root reserves and once ‘used up’ the plant have time to regenerate it's nutrient reserves for further growth, whereas N adds nutrients to the soil profile and plants have an abundance of N available for growth (Davies, 2010).

5.1.6 RPM measurements

However if pasture that has been treated with GA is measured with a rising plate meter (RPM), then caution must be taken as it has been found that through the accelerated growth and upright growth habit of GA treated pasture, RPM DM yield may vary somewhat from actual standing DM yield (van Rossum et al., 2013). Farmers must be able to identify how much pasture is in the paddock so they can make a judgement on when the paddock needs to be grazed. Due to the quick increase in DM yield from GA application, a farmer may need to speed up his/ her round length, or make management decisions based around maintaining pasture quality i.e. shutting up some of the farm for supplement production.

5.1.7 Comparison of DM yield measurements

The study used range of methods to assess DM yield including the lawn mower, quadrat cuts and pasture height. Mower DM yield was deemed to be the most suitable measure in the context of livestock as it represents the amount that may be harvested above a previously grazed horizon, and is a large proportion (%) of the entire plot area. Responses based on mower yield did not always align with those associated with quadrat cuts. For example, in autumn mower DM yield ranged from 1329 to 2227 kg DM/ha between lowest and highest growth responses, whereas quadrat cut yield ranged from 2553 to 3251 kg DM/ha above "O" or ground level for lowest and highest yield. Quadrat cut yields to ground level did not reflect this result. In terms of quadrat cut yield pasture treated with N was the only treatment to give a response; this may have been due to N increasing vegetative material below mower harvest height (< 4.5cm). This was also found by Matthew et al. 2009. The greater response found in the mower yield compared with the response found in the quadrat cut yield may be due to a high level of error associated with taking quadrat cuts. Matthew et al (2009) suggested large errors were to be expected with such a small overall yield response (≤ 500kgDM/ha). They went on to say that to overcome this error a large amount of quadrat cut yield cuts needed to be taken in the small experimental area to overcome the differences expected in a field experiment.

A further explanation of the difference between quadrat cuts and mower yields may be associated with the fact that GA is noted to cause elongation of stems (Brown et al., 1963; Percival, 1980; Whitney et al., 1973). This response was noted in this study where stem elongation was increased (12% (60.5 to 68.5 mm) in Period 1 in autumn and 25% (66.9 to

74.5 mm) in Period 1 in spring) in response to GA, meaning that more herbage was pushed into the mown horizon. Of note is that SSH was increased by GA application, with this response occurring quickly.

5.1.8 Botanical composition

A single application of GA resulted in increased mass of a both ryegrass and white clover in both autumn and spring. When expressed on a percentage of DM basis in Period 1, the percentage of white clover was higher in autumn (33 vs. 29%) and higher in spring (17 vs. 12%) when treated with than without GA. These results are in keeping with previous studies conducted at the same site. Both van Rossum et al. (2013) and Allen (2010) noted at the same site that there was an increase in the white clover in perennial ryegrass white clover pastures. In the study of Van Rossum et al. (2013) the white clover response was greater in ryegrass than tall fescue pastures. They concluded that the effect of GA on botanical composition was dependent on the sensitivity of the base grass to GA application relative to either herbs or legumes in pasture, with tall fescue being more responsive than perennial ryegrass to GA application (Wittwer & Bukovac 1957), and thus out competing the white clover. Allen (2010) went on to say that the ability of GA to lower N content in the pasture and therefore the animals diet was limited due to the increases in white clover content from GA application (18.7% in GA treated pasture vs. 7.4% in N treated pasture). There is little information on GA and the performance of a pure sward of white clover. The results from the current study are in line with those previously found, where GA increased clover DM yield even at the highest application rate of 593g GA/ha (Finn & Nielsen, 1959), whereas grasses treated with GA gave a much higher response at lower application rates of around 70g GA/ha.

The botanical composition response varied between autumn and spring. The different response in autumn may reflect that the grass plants slowed in growth, were more vegetative and had a lower sward height at this time, which allowed the clover to intercept more light and therefore grow more efficiently than in spring where grass plants approached reproduction and, therefore may have had a more upright growth habit limiting the clover plants ability to intercept light (Morgan & Mees, 1958a). Though there was no results to show this, if the application of GA is left later in the season (closer to summer) GA may cause pasture to initiate flowering. This means application of GA should be applied early in spring to alleviate management issues around flowering and maintaining pasture quality

5.1.9 Nutritive value

The effect of GA application on pasture nutritive will depend on the nutritive value of individual species in response to GA combined with how the relative abundance of each species, differing in nutritive value and changes in response to GA application. Samples analysed in this study were mixed herbage samples, collected at mower height, and therefore differences would reflect both changes in nutritive value of individual species and botanical composition. There was little effect of GA on ME; this reflects that GA application did not alter ADF concentration from which ME was calculated (CSIRO 2007). This result is consistent with the study of van Rossum (2013) and of Matthew et al., (2009), where ME remained at 10.9 MJME/ kg DM after 4 weeks in autumn with 24 g GA/ha applied (van Rossum et al., 2013) and remained at 12.5 MJME/ kg DM after 4 weeks in spring with 8 g GA/ha applied (Matthew et al., 2009). This confirms that GA application will have little effect on the quality of herbage harvested by livestock. However, due to the increased ‘stemminess’ of pasture treated with GA, livestock pasture preference may be affected. If swards are not harvested to a low residual due to the increased level of stem, swards may begin to deteriorate in quality over time due to the increased dead material in the bottom of the sward, reduced light interception and therefore growth of clover and a largely unpalatable grass stem, further reducing preference. To maximise herbage production of a ryegrass white clover sward without affecting pasture quality the pasture should be defoliated to between 4 and 8 cm. At a higher residual pasture quality begins to deteriorate because the grass starts to grow seed head and cows prefer a low ADF, high WSC pasture (Lee et al., 2008). Vegetative pasture has these qualities while a reproductive pasture has the opposite, therefore grazing preference is reduced.

There was little effect detected of GA application on CP concentration. This is perhaps surprising given that there was an increase in the proportion of white clover – of high CP concentration – in the pasture with GA application. In part, this increase in white clover may have been offset by a reduction in the CP concentration of perennial ryegrass. In related work (Allen, 2010) which examined the CP content of individual species found that the CP content of the perennial ryegrass in a mixed pasture was decreased by GA application, but this was offset by an increase in the proportion of white clover in the pasture, with little net effect of GA application on CP content of the pasture. The associated effect on CP in the pasture may be enhanced by the effect of GA application on plant growth. In the current experiment CP% was always above 15% (short and long term, and in both seasons); and therefore was adequate for plant growth. In enhancing cool season growth, N uptake from the soil may also be increased, thus minimizing the amount of nitrate that is potentially at risk to nitrate leaching (Allen, 2010). Schofield et al. (1991) predicted that with 1.5% N in the diet 45% of the N was excreted in the urine whereas with 4.0% N in the diet 80% of the N was excreted in the urine. Therefore reducing N content in the pasture with GA application should increase N retained in the animal for production (milk and meat) and reduce N loss in urine (Allen, 2010). The practical implications therefore on the use of GA in late autumn and early spring on a farm is to increase pasture growth and sustain DM intake of livestock, without having an associated increase in the CP content of the diet. This contrasts with N fertiliser. N fertiliser application increased DM yield, with high N content than control. This means N consumed per kg DM is higher which the leads to higher amount of N excreted.

5.1.10 Effect of GA on tiller density

GA may increase the height of pasture via the vertical distribution of dry matter but overall yield may not be increased due to the circumstances of sward thinning, resulting from reduced tiller density. Previous literature found that with increasing level of GA applied average tiller density increasingly decreased (Nickerson, 1959).This supports Blacklow and McGuire's (1971) findings of a tiller mass reduction of European tall fescue variety from 93 to 63 tillers per plant. The results from the autumn experiment support this theory because GA had an effect on tiller density. A single treatment of GA in Period 2 in autumn reduced tiller density by 25%, from 11088 to 8275 tillers/ m^2. Tiller density remained low over the long term response Period (harvest at week 8). However this reduction did not occur in spring, which may be due to the phenology of the plant in spring and the fact that the tillers are dying anyway, and are not replenished until autumn post flowering. Hampton & Hebblethwaite (1984) found that GA applied alone reduced tiller density prior to ear emergence, but afterwards tiller density was similar between GA and untreated pasture (Hampton & Hebblethwaite, 1984). However in this trial autumn and spring responses cannot be directly compared because the trial in spring was located in a different area and in this area N may not have been limiting which may have influenced the response of pasture to GA in regards to tiller density or mass.

N application resulted in increased tiller density in both autumn and spring which has been cited in previous literature (Ball & Field, 1982; Finn & Nielsen, 1959). In autumn N increased tiller density from 8932 to 11547 tillers/ m^2 and in spring a similar increase was found 8875 to 15815 tillers/ m^2. The negative effect of reduced tiller density was overcome when N was applied with GA, as GA + N increased tiller density in both autumn and spring, yielding similar to N applied alone. The practical implication of applying GA to pasture with a sparse tiller population may not be wise. The suggestion would be to apply GA with N to ameliorate the effect of just applying GA by itself.

5.1.11 Future research

The current trial has confirmed previous research findings that GA can in fact increase yield of ryegrass clover pasture in autumn without affecting pasture production in spring and that GA has no adverse effects on pasture ME or nutritive value. Which means GA can help sustainable dairying due to decreased N input in late autumn and early spring which would have otherwise caused nitrate leaching. Further investigation needs to be done on GA and N application and the corresponding N response efficiency in autumn which was found to be high in this experiment. Further research also needs to be done on the effect GA has within a whole farm system, particularly investigating what effect GA has on the profit margin of an operating commercial farm, which will confirm whether GA is not only environmentally sustainable within a farm system but also economically sustainable. Management within a farming system may need to be altered because of the changes GA makes to the sward structure and botanical composition.

5.2 Conclusion

1) A single application of GA increased DM yield of a perennial ryegrass white clover pasture in both autumn (242 kg DM/ha) and spring (343 kg DM/ha). This confirms previous research findings that GA can increase yield of ryegrass clover pasture in the short term.

2) When applied together GA and N gave a very high N response efficiency in autumn, and a normal N response efficiency in spring. The reason for the high N response efficiency in autumn is unknown, further research is needed to find the cause.

3) GA DM yield response was better in a high N environment. GA plus N response was additive and the two complemented each other to achieve the highest DM yield.

4) A second harvest revealed a second application of GA could achieve DM yield increase of 347 kg DM/ha in autumn and 259 kg DM/ha in spring. The lower DM yield increase is thought to be due to changes in temperature and plant phenology.

5) There were no carry over effects from autumn to spring or from Period 1 to Period 2 due to GA application.

6) The combined yield from Period 1 and Period 2 showed that a combination of GA and N followed by either GA (autumn) or N (spring) would give the greatest overall DM yield. The increased DM yield will give farmers feeding options throughout the lactation period.

7) In the long term, GA had little effect on, but did not decrease DM yield which supports evidence from previous literature that GA utilises root reserves for short term growth, so that little or no growth is detected in the long term period.

8) Farmers may need to alter their pasture management strategy when using GA, as RPM may give a true representation of available pasture, and because of the accelerated growth of the pasture farmers may need to make changes to round lengths.

9) GA did not affect root growth.

10) GA has no adverse effects on pasture ME or CP. Growth was still achieved when CP was 15% after GA application.

11) GA increases white clover content in the sward. Because of the increased CP from clover, N content in an animal diet may not change. However GA can still be used as a tool to reduce the negative impact N has on the environment in late autumn and early spring.

12) GA decreases tiller density while N increases tiller density. Caution must be taken when applying GA alone to pasture which has a low tiller population.

Chapter 6 References

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