# Seed growth and development of three perennial ryegrass cultivars after treatment with 'Moddus' straw shortener.

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Master of Agricultural Science
at
Lincoln University

by
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Lincoln University
2012

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# Seed growth and development of three perennial ryegrass cultivars after treatment with 'Moddus' straw shortener.

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First year crops of three diploid perennial ryegrass (*Lolium perenne* L.) cultivars, 'Meridian', 'Bronsyn' and 'Grasslands Impact', that contain the AR1 endophyte, were sown on 1 April and 14 May 2008. A subsequent application of Moddus (a.i. 250 g/l Trinexapac ethyl) plant growth regulator at three rates was used to examine the relationship between seed and stem dry weight in relation to thermal time.

Seed filling of 'Meridian', 'Bronsyn' and 'Grasslands Impact' followed a sigmoidal growth pattern. The lag phase was 150 growing degree days (°C days) and the duration of the linear period constant at 294 °C days. The application of Moddus increased seed yield by approximately 26% for each 800 ml/ha applied from 1715 (0 mlha) to 2195 (800 ml/ha) and 2722 kg/ha (1600 ml/ha). The time to 95% of final seed weight was constant between treatments at 443 °C days. Seed yield increase from Moddus was achieved by increased rate of seed filling per seed head, 0.24 mg/°C days/head, which increased the number of seeds/$m^2$.

For all cultivars, 1600 ml/ha of Moddus produced the highest seed yield and the shortest total stem length. There was a 0.15 m reduction in length between 0 and 1600 ml/ha of Moddus with all internodes shortened, including the seed head length. Stem dry weight increased to a maximum at between 310 and 400 °C days following anthesis. Thus, stems competed with growing seeds from anthesis, throughout the lag phase until approximately 75% of final seed weight. When seed demand for assimilate was low, lag phase and early seed growth, the stem was a competing sink. As seeds developed their sink capacity increased, thus drawing assimilate from the stem. At harvest, stems were 25% heavier than at athesis which suggests they were a net sink for assimilate post anthesis and that there was further assimilates available for seed production.

Moddus also decreased absolute lodging at harvest where stems were horizontal when no Moddus was applied compared with stems leaning on a 45 ° angle for 800 ml/ha and upright for 1600 ml/ha. Moddus increased the harvest index (HI) of plots, 13.5 – 19.8%, and individual stems, 20 – 40%, primarily through an increase in seed mass/ha while straw mass remained constant for 'Meridian' and 'Bronsyn'. In 'Grasslands Impact' the increase in HI was limited through an associated increase in straw DM when Moddus was applied. The change in harvest index per day (dHI/dt) on main stems was linear, which suggests this could be a useful method for incorporation into crop simulation models. The dHI/dt was influenced by treatments which influenced lodging.

Competition for assimilate between stems and growing seeds was a major factor limiting the seed yield. This has implications for plant breeders and seed producers where both should aim to reduce stem length which is likely to increase the rate of seed filling. There was a clear advantage to applying Moddus at 1600 ml/ha for all cultivars which highlighted the advantage of breeding for a shorter stem.

**Keywords:** Assimilate, harvest index, lag, linear, lodging, *Lolium perenne*, Moddus, seeds/$m^2$, seed growth, sowing date, stem weight, Trinexapac ethyl, yield.

# Acknowledgements

I would like to thank my supervisor Professor Derrick J. Moot for the feedback, advice and the various sessions where I was challenged to consider bioloigally what the reuslts in this thesis actually mean.

Thanks to many of the team at the Field Service Center but specifically, Don Heifer and Dave Jack for their technical support in running the field experiment and to Dr Anna Mills for all things related to data analysis, formating and general question and answer sessions.

I would like to thank all those who assisted with the intesive field sampling, commonly three or four times each week including weekends.

Thank you to The Foundation for Arable Research for funding this M.Agr.Sc.

I wish to acknowledge the support of my parents, John and Liz, for encouragement to undertake this period of study, a big thank you for everything to 'mum' whom passed away during the period of this study, we think of you every day.

Finally a special thank you to Tracey, my wife, for your assistance with field sampling and understanding while completing this thesis.  Much family time has been sacrificed in order to complete this project and I thank you for the continued support and patience.

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# Chapter 1

# General Introduction

Perennial ryegrass (*Lolium perenne* L.) is a long lived grass species which is popular in both short and long term pastures in New Zealand. Other end uses include turf grass for stadiums, golf courses, recreation parks and domestic lawns. Perennial ryegrass has the ability to produce a large number of tillers which allows it to fill inter-plant spaces when neighbouring plants are removed, making it ideal for both major end uses. In New Zealand pastures, perennial ryegrass is the benchmark to which other species are compared for dry matter production. Dry matter production varies with climatic conditions and soil fertility but commonly ranges from 10 – 25 t DM/ha (Kemp *et al*., 1999). Seed is the delivery mechanism for improved ryegrass genetics and endophyte status to the pasture and amenity markets.

About 14,000 ha of perennial ryegrass for seed production is grown in New Zealand annually which supplies both the domestic forage and export markets, mainly Australia, Europe and North America. Approximately 85% of the ryegrass seed production occurs in Canterbury where the dry summer conditions facilitate harvesting (Rolston *et al*., 1990).

With an increase in on farm irrigation use creating pressure on land values in the Canterbury and North Otago regions, increases in seed production per hectare have been required to maintain farm viability. The introduction of Moddus (a.i. 250 g/l Trinexapac ethyl ) for lodging control in 1999 gave seed growers a viable method of reducing lodging and the associated seed yield losses, sometimes up to 50% (Rolston *et al.,* 2010a). Recently Trethewey and Rolston (2010) and Griffith (2000) determined stem carbohydrate does not limit seed yield in unlodged crops, but neither authors investigated seed growth in relation to stem dry weight changes in thermal time or between cultivars. Warringa *et al*., (1998) investigated individual seed growth within a spikelet, however only expressed this in days, allowing no transfer of information between sites and seasons.

It is generally accepted that seed growth will follow other monocotyledonous species and have three distinct phases of growth; lag, linear and maturity, which will be better described using thermal time compared with days (Montieth, 1977). The use of thermal time allows for the transfer of information from both seasons and locations.

The objective of this study was to identify the timing of different phases of growth in perennial ryegrass seed and quantify these using thermal time following anthesis while concurrently investigating change in stem weight.  This allows for an understanding of how the stem and seed interact during the seed filling process.

# Chapter 2

# Review of Literature

## 2.1 General introduction

In New Zealand herbage seed production consists of approximately 35,000 ha of production annually. The majority of production consists of perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.), hybrid and Italian ryegrass (*Lolium multiflorum* L). In New Zealand the annual production of perennial ryegrass for seed varies from approx. 12,000 up to 16,000 ha (Pyke *et al*., 2004).

Perennial ryegrass is an important crop in the rotation of New Zealand arable farmers providing a moderate return per hectare (ha), comprised of both grazing/silage and seed production, however seed production provides approximately 85% of the return (Rolston and Archie, 2005). Seed yields from perennial ryegrass in New Zealand have steadily increased up to an average of approximately 1800 kg/ha in 2007, however individual yields of over 3000 kg/ha have been recorded (Rolston *et al*., 2007).

The herbage seed industry returns approximately $80 million to New Zealand annually (Pyke *et al*., 2004). Within New Zealand the herbage seed industry supplies seed for pasture renovation, sports fields, golf courses, parks and domestic use. New Zealand exports seed to over 40 countries with the USA, Australia and Europe representing important markets (Rolston *et al*., 1990, Pyke *et al*., 2004). The development of 'novel' endophytes e.g. AR1 and AR37, which produce insect deterrent toxins e.g. peramine, without the mammalian toxins present as in 'wild type endophytes' have been inoculated into many New Zealand selected cultivars. The endophyte status gives many New Zealand ryegrass cultivars a major point of difference in the international market place (Pyke *et al*., 2004).

An individual perennial ryegrass plant comprises of a collection of tillers which differ in age (Langer, 1979). The variable age structure allows the plant to replace tillers following seed production and maintain its perennial nature, however perhaps more importantly it allows the plant to recover following grazing when individual tillers are removed. Typical thousand seed weight (TSW) for diploid perennial ryegrass is approximately 2 g (Hampton *et al*., 1999).

Other management factors which influence seed production include crop establishment, including sowing date and rate, nitrogen application rates and timing plus defoliation (Langer, 1980). In perennial ryegrass time of harvest is difficult to determine due to a 15 plus day spread in anthesis

(Hill, 1977a). Many authors have defined optimum harvest date by seed moisture content (e.g. Hill, 1977a).

This review covers present knowledge of research on perennial ryegrass seed production with an emphasis on maximising seed yield through pre anthesis management. It includes a section on seed growth post anthesis, assimilate supply and the relationship between stem and seed development post anthesis.

## 2.2 Perennial Ryegrass

### 2.2.1 Botanical description

Perennial ryegrass is a bright to dark green, moderately ribbed and glabrous perennial. The auricles are small and may be shrivelled at their tips, these can be difficult to find. The ligule is short (up to 2 mm long), membranous, light green and often indistinct on small tillers. The leaf blade is 3-20 cm long and 2-6 mm wide. The upper surface is smooth with regular ribs, where the central rib is often positioned lower than others, a rounded to sharp keel is found on the lower leaf surface (Lambrechtsen, 1972). The emerging leaf is folded but may be rolled on older tillers. The base of the leaf sheath is red to purple with the inner leaf sheath pale green.

The inflorescence of perennial ryegrass is a spike capable of producing a variable number of spikelets. Each spikelet is subtended by a single glume, with exception of the terminal spikelet which has two. Each spikelet may contain between 3 and 10 florets (Langer, 1990). The seed of perennial ryegrass is flat, 4-6 mm in length and has an average thousand seed weight (TSW) of either 2 g (diploid) or 2.6 g (tretraploid) following seed cleaning. Each seed consists of a caryopsis surrounded by a lemma and palea, the lemma is awnless (Langer, 1990).

### 2.2.2 Tiller production

Tillers arise from buds in the axil of leaves. The ability to produce many tillers, some of which remain vegetative, provides perennial ryegrass with the perennial characteristic that makes it common among grazed pastures in New Zealand (Langer, 1979). Tiller production is continuous where the rate at which tillers emerge, grow and die varies appreciably dependent on growing conditions and imposed management practices (Hennandez Garay *et al.*, 1997). At any given stage a perennial ryegrass plant comprises a collection of tillers, all differing in age (Langer, 1980). During seed production a portion of tillers remain vegetative, allowing the plant to regrow the following autumn.

Tillering is vital to the perennation of a grass sward, it allows for regrowth when the apical meristem is removed through cutting, grazing or inflorescence development. The regeneration of tillers is particularly important when tillers are lost following seed production or during removal of the inflorescence during cut and carry farm practices e.g. hay making (Jewiss, 1972). Furthermore tillering allows individual plants to fill space if plants in the sward are removed for whatever reason, thus helping to maintain the longevity of a pasture sward. Tillering also aids establishment through the ability to rapidly fill space between plants.

Within a grass tiller the basic repeating unit of growth is a phytomer. Each phytomer consists of a node, an internode, a leaf sheath, a leaf blade and an axillary bud (Moser and Nelson, 1995). Each axillary bud is capable of producing a new tiller. Vegetative tillers have an active shoot meristem which initiates the phytomer components with essentially no internode elongation. After initiation of reproductive development, phytomer production ceases, the apex differentiates into an inflorescence and the internode areas begin to extend. When the internode areas are fully expanded the tiller has reached its capacity for growth and once developing embryos have matured it will potentially die. Production of phytomer components is carried out at the apex in sequence and thus regulates the rate of growth. Each apex has between two and six leaf primordia of various ages. When the inflorescence primordia develop the tiller commits to reproductive development.

In grass species, tillers which die are replaced by new tillers in an organised structure so that plant characteristics and form rarely change. Since each leaf produced has a meristem, potentially a new tiller can emerge from each individual leaf if environmental conditions allow e.g. sufficient water, nitrogen and the correct light environment. This has implications for ryegrass seed crops where young tillers can compete for resources with developing seed during seed filling (refer to section 2.3.7).

## 2.3 General agronomy

This section gives an insight into the general agronomy of perennial ryegrass seed production.

### 2.3.1 Sowing date

In New Zealand's seed producing area, perennial ryegrass has a wide sowing window as long as vernalisation requirements are fulfilled to ensure flowering. However, it has been reported time of sowing influences autumn and winter tiller production, which influences seed production (Hill and Watkin, 1975). Hill and Watkin (1975) showed late sowing (late March/April) reduced fertile tiller number and ear size (presumably spikelet number per ear), however this does not always translate directly into seed yield due to the number of seeds/m$^2$ having the largest effect on seed yield (Langer, 1980).

Rolston and Archie (2005) showed a relative seed yield reduction (P<0.01) of 0.14% per day that sowing was delayed in the autumn, under New Zealand conditions, when trial data from three cultivars and two seasons was combined. However, within individual trials the reduction in seed yield was slow and only the extremes in sowing date were different (P<0.05) from each other. At no stage was there a dramatic reduction in final yield from any of the sowing dates tested. However large differences were recorded in utilised dry matter for grazing, approximately 4500 compared with 0 kg DM/ha for February and May sowings respectively.

Sowing date has the potential to vary considerably from season to season or grower to grower due to management or climatic influences. Delaying sowing affects the date of crop emergence and reduces the amount of solar radiation available for capture during the growing season, this may result in a lower yield if a critical point is passed (Hay and Walker, 1989). The rate of germination and pre emergence growth is determined by soil temperature and sowing depth. When sowing occurs in cool conditions both emergence and leaf appearance rate are reduced. Reduced leaf appearance rate slows the rate of tillering and reduces the number of autumn formed tillers present at the end of winter. Tillering allows for rapid increases in leaf area and is largely determined by management e.g. plant population and nitrogen fertiliser application. It should also be noted that due to competition, large numbers of buds will not develop into tillers or will die prematurely (Hay and Porter, 2006). It has been observed that when tiller number is low at the end of spring, through late sowing, that spring emerged tillers can contribute large percentages of the final seed yield (Rolston and Archie, 2005). This is supported by Hebblethwaite *et al*. (1980) where the number of ears/unit area was the same at harvest when alternate drill rows were removed at double ridge stage to reduce the number of overwintering tillers. The remaining plants were able to compensate with spring emerged tillers and the fertile tiller number at harvest was not reduced. Perennial ryegrass has the ability to be vernalised at the bud stage, meaning tillers which were formed in winter but did not emerge as tillers, can produce mature ears in the spring if fertility and soil water status allow (Heide, 1994, Cooper, 1949). The ability for tillering allows late plantings to reach canopy closure and reduce the losses associated with incomplete radiation interception (Hay and Porter, 2006).

Spring tillering follows a characteristic pattern with a rapid increase in tiller number following the addition of N fertiliser, reaching a maximum around ear emergence. Tiller mortality rate is high in the spring and ultimately the stand would comprise only reproductive heads – however this is not possible or the perennial ability would be lost. Following anthesis the production of vegetative tillers increases (Hebblethwaite *et al*., 1980), particularly in the presence of excess N and soil moisture (Rolston *et al*., 2007a).

Hill *et al*. (1999) presented data which showed the number of spikelets produced per ear was reduced when sowing date was delayed, however they did not state how many harvested seeds were produced by those ears or if any seed yield reduction was expected. Rolston and Archie (2005) stated that seed yield per head was not reduced by later sowing dates but did not present data on spikelets/ear. Overall these data show the ability of individual yield components to vary and it is the final number of seeds/$m^2$ that drives seed production, not the production of any single component.

### 2.3.2 Sowing rate

Sowing rates ranging from between 1 and 20 kg/ha have no effect on the final seed yield of perennial ryegrass through its ability to produce tillers and therefore compensate (Hill *et al*., 1999). The seeding rate for perennial ryegrass seed crops is commonly between 8 and 11 kg/ha (diploid), where over population leads to competition both within and between rows which may reduce establishment (Hill *et al*., 1999). Increased seed rates (20 kg/ha+) leads to competition for space which may result in a number of weak plants with the potential for increased variation across a paddock (Hare, 1990). The ideal sowing rate for seed crops is a rate that gives individual plants the space required to ensure it produces its maximum number of fertile tillers (Hare 1990) which emerge and flower at a similar time, therefore producing an even crop with at least 1800 heads/$m^2$ (Hampton and Hebblethwaite, 1983). Hill *et al*. (1999) therefore suggested 5 kg/ha was an ideal seeding rate for establishing perennial ryegrass seed crops. However when environmental or pest issues e.g. late sowing or slugs are possible, 5 kg/ha may result in reduced populations and increased competition from weeds. Hill *et al*. (1999) showed that at low rates where bird or slug damage was possible final seed yield was reduced as the remaining plants were unable to compensate. Hare (1990) suggested that between 1800 and 2000 ears/$m^2$ are required for high yielding seed crops and that a plant population of 300-400 plants/$m^2$ was required to reach 2000 ears/$m^2$. To establish a stand with 350 plants/$m^2$, with seed quality characteristics of:

- TSW 2.2,
- a germination of 90% and
- an expected field emergence of 90%,

a sowing rate of 8.1 kg/ha would be required to achieve the targeted plant population.  Very few publications mention the use of a target plant populations, perhaps because the plants compensate and approximately 2000 heads/$m^2$ are produced from a wide range of plant densities.

McCloy (2000) compared three sowing rates, 2-3, 4-6 and 8-12 kg/ha at three row spacing.  It was shown that the maximum seed yield (1767 kg/ha) was obtained from 300 mm row spacing and a sowing rate of 8 kg/ha.  However an almost identical yield was shown from 150 mm row spacing and a sowing rate of 6 kg/ha.

In summary it appears that if growers establish an adequate number of plants to produce approximately 2000 heads/$m^2$ at harvest, seed yield of perennial ryegrass will be not be influenced by sowing rate or row spacing.

### 2.3.3 Development of leaf area

The amount of solar radiation intercepted will be determined by the development of a canopy which can intercept all incoming solar radiation (i.e. reach critical leaf area index (CLAI) and therefore grow at its maximum rate).  However, interception depends not only on the amount of leaf area present, (defined as leaf area index, LAI) but also on their distribution and arrangement in space (canopy structure and architecture).  Leaf area index is a measure of a number of plants and not individual leaves, to understand the fraction of intercepted light we must understand the growth and interception of individual leaves and the canopy (Hay and Walker, 1989).

The leaf area of a plant at any given time can be given by a relationship between:

1. Temperature – determining the basic dynamics of crop emergence, leaf and tiller production, leaf expansion and senescence,

2. Nitrogen status – determining the size and duration of leaves, and the development and survival of tillers,

3. Plant population or density – influences early season leaf area development and late season leaf area through tiller competition,

4. Water supply – influences final leaf size and the duration of the crop canopy (Hay and Porter, 2006).

In perennial ryegrass a LAI of about 7 is required to achieve 90 – 95% solar radiation interception ($LAI_{crit}$) compared with white clover where a LAI of about 5 is required. Factors which affect $LAI_{crit}$ include leaf size, shape, thickness and angle. Above $LAI_{crit}$ crop growth rate remains relatively constant with photosynthesis and respiration also constant (Hay and Walker, 1989).

Sowing date has an influence on when leaf area develops. Earlier sowing should allow a crop (and each plant) to develop a larger canopy before the onset of winter. As solar radiation receipts increase in spring a larger canopy earlier will be able to intercept more radiation compared with that of a later sown crop. Since dry matter production is directly related to radiation interception (Gallagher and Biscoe, 1978) the advantage of a larger canopy earlier should lead to a higher yield (at the same harvest index and assuming other factors are not limiting). Since temperature increase in spring often lags behind that of solar radiation, and temperature is the factor which determines LAI expansion, late sown crops usually intercept much less radiation than early sown crops. Since earlier sowing gives individual plants greater time to reach a larger size, sowing rate must be adjusted with delayed sowing date to counteract reductions in maximum individual plant yield. Sowing rate on its own will affect the time period for a canopy to reach $LAI_{crit}$. In cropping situations this may be a disadvantage later in the season (e.g. increased lodging risk) but in a grazing situation inter plant competition can cause an increase in initial plant DM production and earlier grazing (although in mixed swards this may lead to species death).

In practical terms the only way a seed producer can affect the amount of incoming radiation available to a crop is to alter the sowing date of the crop (or the duration of the crop). Sowing earlier in the season will allow greater time for the capture of radiation and the development of a canopy, in effect less radiation should be lost.

### 2.3.4 Nitrogen

Much research has been published on the responses of perennial ryegrass seed crops to the application of nitrogen (Young III *et al.,* 2000, Gislum *et al*., 2007, Rowarth *et al*., 1998, Cookson, 1999, and Rolston *et al*., 2007b). In recent years the recommendations for perennial ryegrass seed production have been remarkably similar in Denmark, Oregon (USA) and New Zealand with optimum responses at approximately 180 - 200 kg of total N/ha (total N is the sum of applied N and measured soil mineral N in late winter – mineral N is the sum of nitrates and ammonium) (Gislum *et al*., 2007; Rolston *et al*., 2010). However most of this work has been agronomic and is of limited use for explaining reasons as to why the difference in seed yield occurs, therefore this section will refer to published information on N and known effects on final seed yield. It is hypothesised that the same 'rules' apply to perennial ryegrass.

Physiologically the application of nitrogen is involved in two critical areas of canopy control, (a) canopy expansion and (b) canopy duration and therefore can have a large influence on the amount of solar radiation intercepted through canopy size and the duration. However, excess nitrogen can also lead to lodging and poor utilisation of incoming incident solar radiation (Rolston *et al*., 2007a).

Plant N status plays an important role in the rate of canopy expansion and on final leaf size (Robson and Decan, 1978). Therefore, nitrogen application early in the growing season is aimed at increasing tillering so that critical leaf area index ($LAI_{crit}$) is reached and the canopy is capable of intercepting maximum solar radiation. Nitrogen has been reported to increase the number and size of leaves (Hay and Walker, 1989). Any increase in the number of leaves is likely to be a result of increased tillering or tiller survival as opposed to an increase in leaf appearance rate, which is dependent on accumulated temperature. There are reports that nitrogen can increase cell size (Hay and Walker, 1989) but the effects are primarily caused by an increase in cell number (i.e. increased cell division) as described by Morton and Watson (1948).

In summary nitrogen applied early in the spring will promote growth which will lead to an increase in tillering and dry matter production. Increased tillering will lead to increased number of fertile ears at harvest (a major yield component) (Hare 1990).

Many authors have shown that nitrogen application is often associated with an increase in head numbers (Hebblethwaite, 1980, Rolston *et al*., 1994, Rowarth *et al*., 1998) and is often accompanied by increasing total dry matter (Cookson, 1999). Increased spring N rate is often associated with increased numbers of vegetative tillers, therefore the ear bearing tillers have to compete with an increased number of reproductive and vegetative tillers for limited resources.

The amount of N fertiliser used has little effect on leaf photosynthetic ability unless nitrogen is limiting (when it affects the amount of Rubisco present in the leaves) (Hay and Porter, 2006), but excess nitrogen can result in large leaves at the top of the canopy resulting in shading (possible light saturation) and premature death of the lower canopy which leads to lower net assimilation rates caused but luxurious growth (Pearman *et al*., 1979).

From a practical perspective it is important to ensure the physiological reasons for strategic N application are not forgotten. The main stems of most species have the ability to produce a number of secondary branches (tillers). This gives a plant the ability to increase the rate of canopy expansion and the final size of an individual plant canopy. Plant population can have a large effect on tiller production as competition among plants for solar radiation means that a large proportion of tillers do not develop beyond the bud stage or die prematurely. Late application of N can cause the survival of ear bearing tillers through to harvest. Achieving a balance between lodging (reduced radiation use and photosynthate supply) and optimum ear numbers is critical to achieving high yields.

### 2.3.5 Defoliation

Defoliation of perennial ryegrass seed crops sown before May is common among seed producers in New Zealand (Rolston and Archie, 2005). Defoliation may occur either by livestock grazing or mechanical means (topping or silage options). Changes in the date of final defoliation before closing for seed production can have large influences on the final seed yield, especially when a large number of stem apices are removed. For example, Hebblethwaite (1983) showed that defoliation after stem extension reduced seed yields by up to 200 kg/ha and that as defoliation was delayed, a greater number of stem apices were removed, which further reduced seed yield. Little work has been completed on identifying the developmental stage for the final defoliation in perennial ryegrass, but the general rule of thumb has been to remove grazing animals before they remove any developing seed heads during stem extension. Recent research in New Zealand has also shown that if the final defoliation date is too early, yield reductions can be large, but not as large as when 20% plus of the ear bearing tiller population are removed (Rolston and McCloy, 2006). Other authors have suggested that defoliation is not required when growing perennial ryegrass seed crops (e.g. Hebblethwaite, 1983, Hampton and Rowarth, 1998, Hare, 1990). The discrepancies in data are likely to be related to the variation in climatic conditions and/or changes in genetics and other management tools e.g. the introduction of the stem shortener, Trinexapac ethyl.

Defoliation too late reduces the number of seed heads through removal of the stem apex (Hill, 1971) – the goal of strategic spring grazing in pasture management. However the disadvantages of defoliation early are not immediately clear. Earlier defoliation gives the crop increased time to produce dry matter compared with those defoliated later. This may lead to an increased risk of lodging, in particular early lodging, while also requiring greater energy reserves for respiration. Rolston and McCloy (2008) suggested the removal of up to 5% of growing points could be advantageous through a more condensed flowering period and even seed ripening, resulting in less seed loss at harvest. Defoliation above apex height reduces the risk of removing the apex and allowing a greater proportion of tillers to reach maturity.

Grazing, in the context of seed production, could be considered as a canopy management tool where the removal of dry matter (leaves) reduces the LAI and lowers the risk of lodging. Grazing has been shown to influence tiller number under various grazing practices from $3 \times 10^3$ tillers/m$^2$ when grazed at 10 week intervals to $20 \times 10^3$ under lenient grazing (Hay and Porter, 2006). In Canterbury, normal closing dates range from late September to early October for mid season flowering cultivars depending on location.

### 2.3.6 Plant Growth Regulators

Plant growth regulators (PGR) have been the focus of much work in perennial ryegrass seed crops (Hebblethwaite *et al*., 1980, Hampton *et al*., 1987, Rolston *et al*., 2004, Chastian *et al*., 2003). The early work revolved around the use of paclobutrazol which showed increases in seed yield of between 8 - 136% (Wiltshire *et al*., 1987). However the chemical properties of paclobutrazol meant that yield reductions in some following crops were possible and therefore its use was not widespread. The introduction of Moddus, (a.i. 250 g/l Trinexapac ethyl), a foliar absorbed PGR, revolutionised grass seed yields in New Zealand with seed yield increases of 50% common (Rolston *et al*., 2004). These results are similar to those achieved by Hebblethwaite *et al*. (1978) where a 61% yield increase was shown through the prevention of lodging using wires. Similar results have been achieved elsewhere e.g. Oregon, USA (Chastian *et al*., 2003) but application rates used by growers are often lower compared with those used in New Zealand. Plant growth regulators are applied with the goal of increasing seed yield through a reduction in stem length, reducing lodging and achieving greater seed set (pollination) and solar radiation use (Hebblethwaite *et al*., 1980, Griffith, 2000).

In New Zealand growers commonly apply between 1200 and 1600 l/ha of Moddus to perennial ryegrass seed crops with the aim of delaying lodging until approximately two weeks before harvest. Moddus activity is optimal when applied at Zadoks growth stage 32 (average of all tillers) (Zadoks, 1974) and inhibits the production of gibberellic acid (Rademacher, 2000). This causes a stem shorting and stem thickening effect, leading to either reductions in total lodging or a delay in lodging (Rolston *et al*., 2007b). Moddus may have an influence on tiller survival (ears/m$^2$) (Borm and van den Berg, 2008), possibly through an improved light environment to avoid tiller starvation (Rolston *et al*., 2007) but does not affect above ground dry weight or spikelet number (Chastain *et al*., 2003). Moddus does affect the number of seeds produced per spikelet through two mechanisms, either (i) increase in floret number (Chastain *et al*., 2003) or (ii) increasing the percentage of florets which produce seed (Rolston *et al*., 2007b), the latter of which is more common in New Zealand. However, limited work has been published which explains why or how a delay in lodging may lead to an increase in seed yield in perennial ryegrass, and those that have, only described lodging as hampering pollination and seed development (Hebblethwaite *et al*., 1978).

### 2.3.7 Lodging

In cereals lodging is associated with grain losses through reduced leaf area duration, variation in grain maturity and increased disease incidence (Hay and Walker, 1989). In perennial ryegrass decreased pollination (Griffith *et al*., 1980), increased vegetative re-growth and lower utilisation of incoming solar radiation (Rolston *et al*., 2007b) can be added depending on the timing of lodging. Pollination is reduced when lodging occurs before anthesis and yields may be reduced by up to 32%, compared with a 15% reduction when lodging occurred two weeks after anthesis (Griffith *et al*., 1980). With the introduction of Moddus to New Zealand, lodging is now more likely to occur between flowering and harvest in seed producer paddocks. For this reason the effects of lodging are more likely to be shown in terms of radiation interception, efficiency of radiation use, increased vegetative growth or some other contributing factor. The use of solar radiation is determined by leaf area index, the arrangement of those leaves (in particular leaf angle to avoid light saturation, extinction coefficient) and the duration of the final yield contributing leaves (Hay and Walker, 1989).

Griffith (2000) investigated the effects of lodging on three cool season grasses, including perennial ryegrass, through the use of poles and wires to support reproductive growth. Lodging reduced ($P<0.05$) seed yield in perennial ryegrass in two seasons through reduced seed numbers and seed weight. Lodging reduced stem weight and inflorescence dry weight by approximately 25% compared with upright plants. Water soluble carbohydrate (WSC) was 30 – 50% less in stems and 36% less in the inflorescence where lodging occurred compared with upright controls. Lodging was associated with a depletion of stem reserves during seed filling while unlodged plants did not show the same decline. When lodging occurs, seeds rely on stored WSC for seed filling as opposed to WSC from current photosynthesis. Seed may have the ability to draw WSC from mid and lower stem regions when secondary growth does not occur (Griffith, 2000). However when secondary tillering occurs new tillers could compete for these assimilates.

When lodging occurs abortion of seed is likely through reduced green area (leaves on buried stems often undergo senescence early) available for the interception of solar radiation and therefore photosynthesis and assimilate supply. When crops are standing very low levels of solar radiation reach ground level or the lower nodes in the canopy.

The timing for the release of daughter tillers is likely to be related to the timing and severity of lodging (Clemence and Hebblethwaite, 1984; Rolston *et al*., 2007b). The release of new tillers (or continued tillering in spaced plants) is determined by availability of solar radiation, more specifically the ratio of red:far red light in the base of the crop canopy (Hay and Walker, 1989). Plants can detect shading through a reduction in the red:far red ratio (Franklin and Whitelam, 2005) where surrounding vegetation can scatter red and far-red light differentially leading to changes in the ratio compared with plants in direct sunlight. A low red:far red ratio reduces the phytochrome photoequilibrium (Pr : Pfr) (Smith, 2000), and has been related to reduced tillering (Barnes and Bugbee, 1991). When lodging occurs, radiation enters the lower canopy, altering the red:far red ratio towards that of a more open canopy, encouraging the release of new tillers. When a new tiller is released from the leaf sheath, it is solely dependent on the main stem for carbohydrate to emerge and produce leaf area. The dependence declines as leaf area increases and the rate of photsynthesis increases (Hay and Walker, 1989; Clemence and Hebblethwaite, 1984).

The release of such tillers shows a loss of apical dominance and will be a competing sink with the developing seed for assimilate stored in the lower stem and internodes.

### 2.3.8 Irrigation

Following the development of a leaf canopy, the major factor affecting the capture of photosyntheticlly active radiation (PAR) is the duration of the developed canopy (leaf area duration, LAD). The maintenance of leaf area up until anthesis is generally carried out by the production of new leaves. Post anthesis, leaf production usually ceases (at least on the tiller/branch where the flowering has taken place), as a result the continued supply of assimilate (photosynthesis) depends of the existing leaf area. Current assimilate production from photosynthesis contributes about 75% of grain yield in high yielding wheat crops (Jamieson *et al*., 1998). The post anthesis period is often critical in terms of supplying assimilate to growing grains as most assimilate produced is used for seed/grain fill (Biscoe and Gallagher, 1977), particularly in annual crops. As a result the persistence of green leaf and stem area during this period can have large implications on the final economic yield achieved.

Weather conditions play an important role in the decline of leaf area after anthesis, in particular temperature and soil moisture status. Biscoe and Gallagher (1977) showed a large variation in the retention of green leaf area for 'Proctor' barley in two contrasting seasons. In 1970 a rapid decline in leaf area index (and faction intercepted radiation) was associated with long periods of bright sunshine, high temperatures and large soil moisture deficit. In contrast green area decline was slow and extended in the 1972 season which was cool and damp.

The same principles apply for perennial ryegrass where Martin *et al*. (2003) showed that when dry matter accumulation was limited by soil moisture, the final seed yield was reduced by 50% when grown in a rain shelter at Lincoln, New Zealand. Variation in final yield was caused mainly through changes in head numbers and final seed size. Although not directly tested the authors estimated that under water stress later formed tillers did not contribute to final seed yield compared with plentiful water conditions where late tillers produced harvested seed.

### 2.3.9 Fungicide application and disease control

The use of fungicides in perennial ryegrass seed production has become standard practice with average yield responses of approximately 20% (Rolston *et al*., 2004). Stem rust (*Puccinia graminis*) reduces green leaf area duration and is particularly devastating when infection occurs on the seed head with yield increases of up to 94% reported by FAR (2006) from the best fungicide treatments. In this trial the increase in seed yield was highly correlated to increased green stem area and green leaf area retention. Stem rust affects radiation use efficiency (RUE) through damaged tissue intercepting radiation that cannot be used for photosynthesis. Diseases utilise WSC produced by the host plant as an energy source, therefore less WSC is available for seed or dry matter production. Infection prior to anthesis can reduce the build-up of stem reserves prior to the beginning of seed development (Griffith, 2000).

### 2.3.10 Harvest index

The harvest index (HI) of perennial ryegrass is variable and commonly ranges from 10 - 20% of above ground mass (Martin *et al*., 2003, Rolston *et al*., 2007b) while the harvest index for most intensively cultivated small grain crops range from 40 – 60% (Hay, 1995). Harvest index is a measure of the proportion of total dry matter of a crop which represents economic yield (Equation 2.1) and as such has a value between 0 and 100% (Hay 1995).

Equation 2.1 HI = grain yield (or weight) / total plant DM (weight)

Harvest index effectively deals with the economic component of yield e.g. seed in perennial ryegrass seed crops, and is often associated with assimilate or dry matter partitioning. The economically important component of seed crops depends on the translocation of assimilates from the leaves or other photosynthetic tissues (Hay and Walker, 1989) unless that component can fix 100% of its own assimilate. Different plant organs are often divided into either sink or source organs. The stem shortening plant growth regulator Moddus, increases the harvest index of perennial ryegrass seed crops through increasing the amount of harvested seed while maintaining the same amount of non seed biomass at harvest (Rolston *et al*., 2007b). Changes in harvest index are often a result of changes in the source or sink mechanisms. Management strategies e.g. closing date which change the amount of crop dry weight often affect seed yield and show a small change in harvest index. Nitrogen application often increases the amount of crop mass while showing very small changes in harvest index (Cookson, 1999). In wheat harvest index has increased from about 30% to 50% in the 20$^{th}$ Century, primarily through an increase in grain population density associated with the introduction of the *Rht2* dwarfing gene (Hay, 1995). Increases in harvest index in perennial ryegrass are also likely to be as a result of increased seed population density (Hebblethwaite, 1980).

### 2.3.11 Change in harvest index over time

The rate of change in harvest index over time could be useful for modelling harvested seed yield. If total dry matter is known, or modelled and the change in harvest index constant, then seed yield at any point following anthesis can be estimated. The rate of change in harvest index (dHI/dt) has been shown to be constant in maize (*Zea mays* L) (Muchow *et al*., 1990) sorghum (*Sorghum bicolor* L) (Muchow, 1988) and wheat (Moot *et al*., 1996). Moot *et al*. (1996) demonstrated low variability for dHI/dt among different wheat cultivars, fertility and drought treatments suggesting that dHI/dt is relatively stable, making it desirable for modelling crop growth. A linear change in harvest index allows for the calculation of seed yield at any point following anthesis, where daily crop mass is known, through multiplying the daily mass by the daily/thermal time value of harvest index. It also allows for the calculation of daily seed growth rates.

No published data was found where dHI/dt in perennial ryegrass was presented.

## 2.4 Thermal time

Thermal time is based on the principle that many crop phenological and growth processes occur in direct relation to accumulated temperature (Monteith, 1977). Accumulated temperature can be known as the amount of physiological time experienced by a plant and can be used to estimate the duration between stages of development. Below a critical temperature crop development stops, known as base temperature ($T_b$), and thermal time accumulation ceases. Thermal time, in its simplest form can therefore be calculated by Equation 2.2:

Equation 2.2 $$\text{Thermal time} = [(T_{max} + T_{min})/2] - T_b$$

Where $T_{max}$ is maximum daily temperature,

$T_{min}$ is minimum daily temperature,

$T_b$ is base temperature, below which development stops (Montieth 1977).

## 2.5 Reproductive initiation

Development towards anthesis and seed production in perennial ryegrass is driven by exposure to cool temperatures (vernalisation) followed by lengthening days (Cooper, 1949). In perennial ryegrass the required temperatures for vernalisation range from between 0 and 14 °C, while the period for exposure varies within cultivar from between 2 to 12 weeks at temperatures of approximately 4 °C (Evans, 1960). Only when vernalisation requirements have been meet can perennial ryegrass respond to long days. Critical day length is dependent on cultivar with variation ranging from 9 to 15 hours (Hurley *et al*., 2006) and is often related to the environment in which it was selected (Heide, 1994). Photoperiod is perceived by the protein phytochrome which is found in the leaves. Phytochrome provides a biological clock through its ability to change form during daylight and darkness, therefore providing a ratio for the detection of photoperiod (Volenec and Nelson, 2003).

The first visual indication of spikelet development is the appearance of double ridge. The spikelets in the middle of the spike are the first to differentiate as for wheat (Kirby and Appleyard, 1981) followed by those towards the base and the tip (Cooper, 1949). The differentiation of each spikelet structure begins first in those spikelets with the greatest elongation (the middle of the ear) and progresses within remaining spikelets basipetally and acropetally, the terminal spikelet is the last to differentiate.

## 2.6 Pattern of anthesis

Anthesis begins in the proximal florets of the central spikelets and progresses both basipetally and acropetally, however in contrast to wheat and barley the spikelets closer to the tip of the ear flower earlier than the basal spikelets. Results from Warringa *et al*., (1998) showed that anthesis started in the proximal florets of the central spikelets, while spikelets closer to the top of the ear flowered earlier than the basal spikelets. Within a spikelet flowering started at the base and proceeded to the tip at a rate of about 1.1 -1.2 florets per day. The distal florets of the basal spikelets were last to reach anthesis about 15 days after the onset of anthesis. The ripening of the set seed generally followed the same trend as the flowering pattern. Moisture content of seeds in the upper spikelets decreased ($P<0.001$) first followed by the basal spikelets. This was influenced by the rate of moisture loss which was faster ($P<0.012$) in the upper spikelets compared with the basal spikelets (Warringa *et al*., 1998). Warringa *et al*. (1998) showed that the average growth rate of seeds did not differ between spikelets, however the growth duration (defined as time between anthesis and maximum seed dry weight) increased from the upper spikelet (24.7 days) to the basal spikelet (30.2 days). Within a spikelet the water content of the proximal, central and distal seed started to decline simultaneously and at a similar rate, 30 days after the onset of anthesis (Warringa *et al*., 1998).

As a result of the flowering and ripening pattern, 4% of the variation in seed dry weight was accounted for between spikelets while 89% variation was explained by seed dry weight within spikelets. Within spikelets seed weight ranged from 1.86 mg in the proximal seed to 0.71 mg distal seed. Thus, the reduction in seed size within a spikelet was a function of reduced growth duration and growth rate (of greater importance). The shorter duration of growth is attributed to the distal and central florets within a spikelet flowering later than proximal florets but ripening occurs simultaneously (Warringa *et al*., 1998).

Within the studies by Warringa *et al*. (1998) all attributes of seed growth were expressed as days after anthesis which makes it difficult to transfer the results between locations and seasons. The final seed weights were low compared with those expected in New Zealand. Maximum seed weight was 1.86 mg. In New Zealand perennial ryegrass generally expected to have individual seed weight of 2.0 mg or a thousand seed weight of 2.0 g. This result may be caused by variation in genotypes, location or management/environmental interactions.

## 2.7 Seed growth

The final phase in crop development is the period from anthesis to physiological maturity. Seed development begins with fertilisation commonly known as flowering or anthesis in monocotyledonous species. Seed growth has been examined in perennial ryegrass by Warringa *et al*. (1998) and in detail for wheat by Loss *et al*. (1989). There are three main phases during the development of a seed:

1. Cell division and expansion
2. Physiological maturity
3. Dry down.

Phase one is where approximately 80% of the growth occurs. During this time rapid cell division occurs followed by an influx of water to drive cell expansion, leading to a rapid increase in whole seed fresh weight and water content. This period is often referred to the lag phase when seed development is expressed in terms of change in dry weight. A period of linear weight gain follows as the result of the synthesis and deposition of stored reserves. Throughout this period the fresh weight remains relatively unchanged while dry weight increases as water is displaced by the accumulating insoluble reserves within the storage tissues. The decline in water content slows as the seed approaches its maximum dry weight. At maximum dry weight the seed is considered physiologically mature and is usually viable. The final phase is the dry down period where moisture is lost to the environment and seed may be shed from the ear depending on the species. Limited work has been completed previously on perennial ryegrass in relation to changes in seed moisture content, dry weight, changes in seed head colour, germination and endosperm consistency. This work has been expressed relative to days after peak anthesis (e.g. Hyde *et al*., 1959, Anslow *et al*., 1964 and Hill and Watkin, 1975). Based on results for wheat, Monteith (1977) concluded that kernel filling should be related to thermal time rather than days from anthesis.

### Grain growth parameters in wheat

The use of thermal time has been successfully used to estimate the time from anthesis until maturity in cereals, where accumulated temperature has been shown to determine the grain filling period. Grain growth in wheat has three recognised phases spanning approximately 800 °C days. These can be estimated using computer simulation, e.g. Sirius (Jamieson *et al*., 1998) where time from anthesis until maturity is split into three phases,

1. Lag phase, anthesis to the beginning of rapid grain fill, this is based on one phyllochron (100 °C days) where thermal time is calculated based on canopy temperature (Jamieson *et al*., 1998) (100 °C days Loss *et al*., 1989).

2. Grain filling, typically based on 550 °C days (base temperature 0 °C) with thermal time based on air temperature (Loss *et al*., 1989).

3. Dry down, an interval of 200 °C days for the grain to dry down after the end of grain filling (Jamieson *et al*., 1998).

Data supporting the use of thermal time on cereals in relation to seed development has also been presented by Loss *et al*., (1989) and reviewed by Hay and Kirby (1991). The proportion of grain filling duration estimated to be lag phase by Loss *et al*., (1989) ranged by 6-18% and was positively correlated with the duration from sowing to anthesis. Physiologically the lag phase is characterised by rapid cell division until the potential cell number (and therefore the potential grain size) is reached (Brocklehurst, 1977). During the linear phase of growth, starch is deposited in the endosperm and the grain takes on a milk-like consistency followed by dough-like consistency. During this phase the rate at which dry matter is deposited in the grain is usually considered constant (Loss *et al*., 1989). The final phase begins when lipids are deposited in the phloem strands supplying assimilate to the grain and grain growth ceases. Physiological maturity is referred to as the time when maximum dry matter is achieved. Maximum growth rates can occur up until physiological maturity or it may decline as physiological maturity is approached. Chalabi *et al.,* (1988) found that is some cases the kernel growth rate fell by 66% from its maximum before growth ceased. After physiological maturity, the water content of the grain decreases at a rate determined by the atmospheric conditions to a level where safe harvesting and storage can occur (Evans *et al*., 1975).

## 2.8 Source - sink relations

Often an understanding of plant physiology is used to determine if crop yield, under a certain set of circumstances, has been limited by the capacity of green tissue to generate assimilate (source limitation) or by the capacity of the harvested organs to store it (sink limitation). Organs are commonly defined as either source or sink organs when the transport of assimilate is discussed. An organ is reported as a source or sink depending on the direction of net assimilate transport e.g. net exporter of assimilate equals 'source'. Leaves are often referred to as source but start life as a sink when they are immature and not fully expanded. During leaf development, tissues for the export of assimilate are developed and at a certain development stage the leaf becomes a net exporter of assimilate (Hay and Walker, 1989). Mature leaves form the main source of assimilate from current photosynthesis, however assimilate can be stored for later remobilisation.

Up until anthesis there are three major sinks for assimilate; initiated leaves that are not yet mature, developing stem/sheath tissue and reserve accumulation in the stem or other tissue. After anthesis, the remaining leaves and green ear act as the source for current photosynthate while the stem and developing grains are sinks. However, during later stages of grain filling there is usually transport of assimilate from the stem to growing grains when the photosynthetic tissue has undergone senescence. The competitiveness of a sink (ability to attract assimilate) depends on its relative strength and its distance from the source. A weak sink can attract assimilate when located adjacent to a source, at the detriment of a stronger more distant sink (Hay and Porter, 2006).

The relationship between sink and source has often been investigated using treatments which affect the supply of assimilate per grain e.g. shading, defoliation, thinning or ear manipulation (Bingham *et al*., 2007). Many of the ear manipulation (removal or sterilisation) treatments have found limited increase in the remaining grain weight. Where increases are shown, they are rarely of the magnitude imposed by the treatment which altered the source sink ratio (Bingham *et al*., 2007). Hay and Porter (2006) stated that in general, source and sink are not independent and such treatments are inappropriate for the investigation. The authors however support a method using grain population density analysis to investigate if yield is source or sink limited. In summary if growing conditions allow for adequate ear and grain formation then yield is likely to be source limited, however if stress occurs during reproductive development then these crops are likely to be sink limited.

### 2.8.1 Source sink relations following anthesis in *Lolium* species

In *Lolium* species it appears the developing head competes with the elongating stem for assimilate during stem extension (Ryle, 1970). During stem extension, the stem is using assimilate to build structural components which will ultimately give rise to a mature seed head. Concurrently the developing embryo head is itself undergoing spikelet differentiation and building the sites for seed growth. In this scenario it is likely the stem is the stronger sink for assimilate, therefore the developing head is potentially source limited. If the developing head is source limited during its development this could lead to less seed sites available for seed production following anthesis. Warringa and Marinissen (1997) showed that the stem was a strong sink during stem extension and used more assimilate than it could produce itself. Therefore assimilate was imported from leaf tissue to sustain stem extension, possibly to the detriment of the developing seed head.

On a reproductive tiller, the younger, upper leaves supply carbohydrate to the upper stem and seed head while the older lower leaves mainly supply the lower stem, young tillers and the root system (Clifford and Langer, 1975; Clemence and Hebblethwaite, 1984). After ear emergence the seed head becomes the main source organ on the flowering tiller with its relative importance increasing as the leaves age (Clemence and Hebblethwaite ,1984). This is supported by finding of Warringa and Marinissen (1997) who showed the source supplied from leaves reduced from 95% of total at anthesis to 16% at seed maturity. In this case the ear became the main assimilating organ.

Clemence and Hebblethwaite (1984) showed that the percentage of labelled $^{14}C$ exported to younger tillers increased from 10 - 24% during seed development while concurrently export to the ear increased from 7 – 34% within 24 hours of application. Colvill and Marshall (1984) also demonstrated export of current assimilate when 16% of label-C was exported to young tillers and 50% to the seed head at seed maturity. While both studies may not agree on the proportion of assimilate distributed to younger tillers, both demonstrate that assimilate is translocated from the stem into seed following anthesis.

During anthesis the stem is a stronger sink than the seeds, consequently carbon from the leaf activity is imported by stems and not seeds. Warringa and Marinissen (1997) showed that 70% of $^{13}C$-label applied at anthesis remained in the stems at harvest. They also demonstrated the stem remained a net importer of assimilate up until about mid seed filling. However after mid seed filling applied $^{13}C$-label was transported from stem and leaf tissue into the developing seeds. This is supported by the results of Clemence and Hebblethwaite (1984) who also described the stem as changing from a sink to source during 'mid seed filling' period.

To summarise; during stem extension the stem is a strong sink for assimilate, possibly depriving the seed head during its development. The stem continues to be a strong sink until approximately mid seed filling when the seed sink becomes strong enough to attract assimilate, or the stem has reached its capacity (which no author has mentioned).

The overall aim of this experiment was to investigate and quantify parameters required to describe perennial ryegrass seed growth and stem relations post anthesis. At the same time agronomic responses of seed yield were obtained for comparison to published data. It is hoped that improved understanding of the processes that occur post anthesis could guide plant breeders to find selection traits that increase seed yield.

# Chapter 3

# Materials and Methods

## 3.1 Experimental site

The experiment was in paddock 9 of Iversen field at Lincoln University, Canterbury, New Zealand (43°38'S, 172°28E). The soil type is a Wakanui silt loam with 1.8 – 3.5 m of fine textured material overlying gravels (Cox, 1978). The previous cropping history was one year barley (*Hordeum vulgare* L.), preceded by four years of lucerne (*Medicago sativa* L.).

Following harvest of the barley crop, a 150 mm soil test was taken (Table 3.1). The area was subsequently ploughed and rolled to produce a stale seed bed. Volunteer barley was controlled using Lion (active ingredient (a.i.) 360 g/l glyphosate) at 3 l/ha. Prior to planting the area was topworked using a tyne cultivator followed by harrow and a 'Cambridge roller' to produce a fine but firm seed bed.

Table 3.1. Pre plant soil test results for paddock Iversen 9, March 2008.

| pH | Olsen soluble P (ug/ml) | Calcium (me/100g) | Magnesium (me/100g) | Potassium (me/100g) | Sodium (me/100g) |
|---|---|---|---|---|---|
| 6.1 | 24 | 6.4 | 0.94 | 0.85 | 0.20 |

The experimental design was a split, split plot with three replicates. The main plot treatment was sowing date (1st April and 14th May 2008), sub plot treatment was cultivar ('Meridian', 'Bronsyn' and 'Grasslands Impact') and the sub - sub plot treatment was rate of the plant growth regulator Moddus® (a.i. 250 g/l Trinexapac ethyl) (0, 800 and 1600 ml/ha). All ryegrass cultivars were sourced from Agriseeds NZ Limited and had been harvested approximately 3 months prior to the commencement of this experiment. All cultivars contained the AR1 endophyte. Individual plots were 2.1 m wide by 10 m long and sown with an Øyjoord cone seeder in 150 mm rows.

'Meridian' is a true perennial ryegrass selected out of a cross between 'Kangaroo Valley', an Australian ecotype, and 'Yatsyn 1'. 'Meridian' reaches anthesis 17 days earlier than 'standard' cultivars and was first entered into the Seed Certification Scheme in 1998. ‘Bronsyn’ reaches anthesis at the ‘standard’ time and is a selection from a ‘Mangere pasture’ and shows characteristics of a pure perennial ryegrass (Agriseeds – personal communication, September 2012). Bronsyn was first entered into the Seed Certification Scheme in 1997. ‘Grasslands Impact’ reaches anthesis 21 days later than ‘standard’ cultivars. ‘Grasslands Impact’ was selected from crossing late heading plants selected out of ‘Grasslands Nui’ with an ecotype imported from North West Spain. ‘Grasslands Impact’ was breed as a true perennial, but was classified as a ‘long rotation hybrid’ due to containing a low percentage of tip awning on the seed (S. Easton – personal communication, September 2012). ‘Grasslands Impact’ was first entered in to the Seed Certification Scheme in 1992.

### 3.1.1 Sowing

The target sowing depth was 15 mm followed by tyne harrows attached to the drill. Plots were ‘Cambridge rolled’ the same day as planting. Sowing rate (Table 3.2) differed according to germination percentage, thousand seed weight (TSW) and the expected field emergence. Sowing rate was calculated to obtain a target plant population of 300 plants/$m^2$ (Equation 3.1). Germination percentage was determined by a germination test carried out to International Seed Testing Association (ISTA) standards by Agriseeds Ltd. Between sowing dates, seeds were stored in a chiller at 4 °C (not humidity controlled). The expected field emergence used for the April and May sowing dates were 90 and 75%, respectively.

**Table 3.2.** Thousand seed weight (TSW), sowing rates and expected field emergence (%) of perennial ryegrass cultivars sown on two dates at Lincoln University, Canterbury in 2008.

| Cultivar | Thousand seed weight (g) | Sowing date one 1 April (kg/ha) | Sowing date two 14 May (kg/ha) |
|---|---|---|---|
| ‘Meridian’ | 2.45 | 7.8 | 9.4 |
| ‘Bronsyn’ | 2.55 | 8.8 | 10.7 |
| ‘Grasslands Impact’ | 2.20 | 9.3 | 11.2 |

Equation 3.1

$$\text{Sowing rate (kg/ha)} = \frac{\text{Target plant population} \times \text{TSW} \times 100}{\text{Germination (\%)} \times \text{Emergence (\%)}}$$

### 3.1.2 In crop management

Within two days of each sowing, plots were sprayed with 1.5 l/ha Nortron® (a.i. 500 g/l ethofumesate) as a pre-emerge treatment for the control of annual grassweeds. Broadleaf weed control was achieved by one spray of 1.5 l/ha Jaguar® (a.i. 25 g/l diflufenican and 250 g/l bromoxynil) on the 20th May (both sowing dates). Further weed control of *Poa annua* was achieved by hand grubbing in mid spring.

Each treatment was defoliated using a ride on lawn mower as required to keep vegetative yields of dry matter present below approximately 3500 kg DM/ha (the limit at which the lawn mower could cut). Each defoliation was cut to a height of approximately 60 mm. The final defoliation occurred when approximately 5% of tillers removed had nodes present. This was equivalent to Zadoks growth stage 30, (as an average of all tillers) (Table 3.3).

Moddus was applied at 0, 800 or 1600 ml/ha at Zadoks growth stage 32 (Zadoks, 1974) for each cultivar (Table 3.3) in a mix with 200 ml/ha Proline® (a.i. 250 g/l prothioconazole) for early season disease control. An average Zadoks growth stage of 32 was the mean of 50 tillers when dissected lengthwise and nodes visually assessed. Dissections were carried out each 3 days during this time frame. Application was made through a motorised boom sprayer at 200 l of water/ha using 04 flat fan nozzles at 300 Kilopascals (Kpa) of pressure.

**Table 3.3**. Final defoliation date, Moddus application date and the days between final defoliation date and Moddus application for all main plot treatments of three perennial ryegrass cultivars sown on two dates at Lincoln University, Canterbury in the 2008/09 growing season.

| Sowing date | Cultivar | Final defoliation date | Moddus application date | Days from defoliation to Moddus application |
|---|---|---|---|---|
| 1 April 2008 | 'Meridian' | 6 September | 26 September | 20 |
| | 'Bronsyn' | 22 September | 15 October | 23 |
| | 'Impact' | 7 October | 21 October | 14 |
| 14 May 2008 | 'Meridian' | 29 September | 15 October | 17 |
| | 'Bronsyn' | 7 October | 23 October | 16 |
| | 'Impact' | 15 October | 28 October | 13 |

Nitrogen was applied in a split of three applications based on nodal development with a target N application rate of 180 kg/ha (Table 3.4). Soil mineral N was measured using a soil spear to a depth of 600 mm in 300 mm increments on 1 September. Soil mineral N is the sum of the plant available forms of ammonium and nitrate as determined by laboratory tests (for this experiment the Hill Laboratories in Hamilton was used). The first N application of 50 kg/ha occurred on 10 September as growth commenced in spring and was applied as Calcium Ammonium Nitrate (CAN, 27% N) using a tractor mounted boom spreader. All subsequent applications were applied by hand. The second N application occurred at the final defoliation and the third N application approximately three weeks later, both as Urea (46% N). The date of final defoliation was determined by nodal development. The three cultivars have different ear emergence dates which lead to variation in defoliation dates and therefore N application dates. The timing of N was aimed to avoid inducing rapid growth during early stem extension which leads to early lodging.

**Table 3.4.** Nitrogen application dates and application rates for three cultivars of perennial ryegrass sown on two dates at Lincoln University in the 2008/09 growing season.

| Sowing Date | Cultivar | Nitrogen application rate (kg N/ha) and application date. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 10-Sept* | 29-Sept | 7-Oct | 16-Oct | 23-Oct | 11-Nov | 14-Nov | 18-Nov | Total |
| 1 April | 'Meridian' | 50 | 75 | 55 | | | | | | 180 |
| 1 April | 'Bronsyn' | 50 | | 75 | 55 | | | | | 180 |
| 1 April | 'Impact'** | 50 | | | 75 | | | 55 | | 180 |
| 14 May | 'Meridian' | 50 | 75 | 55 | | | | | | 180 |
| 14 May | 'Bronsyn' | 50 | | | 75 | | 55 | | | 180 |
| 14 May | 'Impact'** | 50 | | | | 75 | | | 55 | 180 |

Note *= as Calcium Ammonium Nitrate (27% N, 8% Ca) all other applications as Urea (46% N).
**= 'Grasslands Impact'

Disease control was achieved through two further applications of Proline, 400 ml/ha, at head emergence and mid flowering. Kocide 2000DS (a.i. 350 g/kg copper) was applied for the control of bacterial blight in 'Meridian' and 'Bronsyn' (Table 3.5). Atomic (a.i. 105 g/litre epoxiconazole and 420 g/litre carbendazim) was applied to limit secondary spread of blind seed disease in all cultivars on 19 December. All applications were made through a tractor mounted boom sprayer delivering 200 l of water per ha using 04 flat fan nozzles at 300 Kpa pressure.

**Table 3.5.** Fungicide product, application rate and application date for three perennial ryegrass cultivars sown on two dates at Lincoln University, Canterbury in the 2008/09 growing season.

| Sowing date | Cultivar | Application dates, rate and product | | | | |
|---|---|---|---|---|---|---|
| | | 21-Nov | 10-Dec | 17-Dec | 19-Dec | 31-Dec |
| 1 April | 'Meridian' | 400 ml/ha Proline® and 1 kg/ha Kocide® | 400 ml/ha Proline® | | 600m/ha Atomic | |
| | 'Bronsyn' | 400 ml/ha Proline® and 1 kg/ha Kocide® | 400 ml/ha Proline® | | 600m/ha Atomic | |
| | 'Grasslands Impact' | | | 400 ml/ha Proline® | 600m/ha Atomic | 400 ml/ha Proline® |
| 14 May | 'Meridian' | 400 ml/ha Proline® and 1 kg/ha Kocide® | 400 ml/ha Proline® | | 600m/ha Atomic | |
| | 'Bronsyn' | 400 ml/ha Proline® and 1 kg/ha Kocide® | 400 ml/ha Proline® | | 600m/ha Atomic | |
| | 'Grasslands Impact' | | | 400 ml/ha Proline® | 600m/ha Atomic | 400 ml/ha Proline® |

Irrigation was applied as required to ensure water stress was eliminated. A water budget using accumulated evapotranspiration (ET) minus rainfall was used to determine water deficit. A trigger point for irrigation was set at 80 mm deficit (sum of ET minus rainfall). Actual water use was measured using a Neutron Probe as irrigation trigger point was approached to ensure crop access to water. Irrigation was applied using either a travelling rotating boom or small 'hard hose big gun' at a rate of 50 mm per application. Evapotranspiration was recorded from a National Institute for Atmosphere and Weather (NIWA) weather station located at Broadfields approximately 3 km North of the experimental site. Rainfall and irrigation applied were recorded at the experimental site using two rain gauges located within the experiment.

Water use differed with the maturity length of each cultivar where 'Meridian' and 'Bronsyn' had four irrigation applications compared with five for 'Grasslands Impact' (Table 3.6).

**Table 3.6.** Irrigated timing, amount (mm) applied and the potential soil moisture deficit (PSMD) when application occurred for three cultivars of perennial ryegrass grown at Lincoln University, Canterbury in the 2008/09 growing season.

| Irrigation date | amount applied (mm) | PSMD (mm) |
|---|---|---|
| 11 November | 48 | 79 |
| 29 November | 19 | 91 |
| 6 December | 52 | 102 |
| 10 December | 50 | 65 |
| 9 January* | 48 | 66 |

* Treatment applied after 'Meridian' and 'Bronsyn' had been harvested.

### 3.1.3 Anthesis

Plot anthesis was assessed on all plots each 2-3 days. A visual assessment of 20 stems was carried out four times at random within each plot (combined 80 stems per plot). A stem was determined to have reached anthesis when at least a single floret was open for pollination (Photo 1). For main stem anthesis, 20 main stems were tagged on 12 September with red paint. Paint was replaced with wire tags ≈ 10 days post final defoliation. Main stem anthesis was assessed using the same criteria on the 20 tagged stems.

**Plate 1.** Anthesis on a single spikelet of perennial ryegrass (*Lolium perenne* L.), cultivar 'Meridian', 11 November 2008. Sown 14 May and treated with 800 ml/ha Moddus at Lincoln University, Canterbury, in the 2008/09 growing season.

### 3.1.4 Seed harvest

At 40% seed moisture content (determined on hand collected samples by oven drying at 130 °C for one hour) three rows by 1 m in length were cut at ground level from two locations within each plot to obtain a harvest seed yield and above ground dry matter. Within each quadrat two 300 mm sections of row were removed for harvest component analysis (see section 3.1.5) and then returned to the bulk sample for seed yield analysis. Cut samples were placed in hessian bags and allowed to dry naturally by hanging over an outdoor drying rack. After approximately 14 days bags were collected and stored indoors. Three months following harvest, samples were threshed using a Winterstieger stationary thresher with all seed collected for further processing. The straw was weighed and a sub sample (approximately 200 g at 15 % moisture) collected for oven drying to determine DM percentage for calculating straw mass at harvest. The seed was cleaned to a first generation seed certification standard using a screen cleaner for the removal of straw. Using a

Dakota seed blower seed was then sorted into fractions where individual seed was either lighter or heavier than 1.5 mg. To confirm seed weights, 200 individual seeds from each sorted treatment were weighed to four decimal places. Seed greater than 1.5 mg was classed as 'first grade' seed which was considered heavy enough to be saleable. Seed below 1.5 mg was re-cleaned to remove empty lemma and paella factions to achieve an individual seed weight ranging from 0.5 – 1.5 mg. This was considered 'second grade' seed. All seed yields are reported at 11% seed moisture content.

### 3.1.5 Harvest components

Seed head numbers were determined by counting the number of heads present in two 300 mm sections of row from two locations in each plot. The two samples were averaged and multiplied up to per $m^2$. Stem length, internode length and the number of spikelets/head were measured/counted on 50 stems from each sample (100 stems/plot). Thousand seed weight was determined by counting 500 seeds from the final machine dressed sample and multiplying by two.

### 3.1.6 Seed growth - changes in head and stem weight

At, and following, anthesis a 300 mm length of row across three drill rows was cut at ground level each 3-4 days until maturity. At each harvest 20 main stems were dissected into two components, stem and head, by cutting at the basal node on the seed head. The leaf blade and any sub tending tillers were removed from stems. Heads and stems were then oven dried at 60 °C until a constant dry weight was achieved. The remaining sample weight was oven dried to obtain changes in dry matter accumulation. Following anthesis any change in head weight was assumed to be that of seed growth. To confirm this heads at the final harvest were dissected where spikelets were removed from the rachis and the rachis weight compared with that at anthesis.

### 3.1.7 Statistical analysis

All statistical analysis was performed using Genstat 14. All plot data were analysed using an analysis of variance (ANOVA) with means separation achieved using the least significant difference (LSD) test. For all linear and non-linear regression, all individual plots were fitted in Genstat 14 to generate appropriate parameters. If all plots responded in the same way treatments were combined across reps for curve fitting, further information is provided within the respective sections for regression analysis.

## 3.2 Climate

The 2008/09 winter (June, July and August) was wetter than the long term average with 325 mm of rainfall compared with the long term mean of 190 mm. Spring (September, October and November) was drier than the long term mean with 75 mm of rainfall compared with the long term mean of 145 mm (Table 3.7). Temperatures were similar to the long term mean for all months. Total solar radiation receipts were similar to the long term mean for most months, albeit slightly greater during October and November where 1250 MJ/m$^2$ were received compared with the long term mean of 1150 MJ/m$^2$.

The increased winter rainfall caused issues with water logged soils and flooding in the particular paddock used for this experiment. This required the installation of drainage and the use of pumps to remove excess water in both July and August. This caused a minor loss in plant numbers, approximately 1%. These areas were avoided during sampling.

**Table 3.7.** Weather parameters recorded throughout the duration of the experiment from 'Broadfields' weather station. Long term means (1975-2004) in parenthesis.

| Month 2008/09 | Rainfall (mm) | | Air temperature Maximum (°C) | | Air temperature Minimum (°C) | | Solar Radiation (MJ/m$^2$) | |
|---|---|---|---|---|---|---|---|---|
| April | 30.6 | (48.1) | 17.2 | (17.7) | 7.2 | (6.8) | 277 | (287.9) |
| May | 64.6 | (49.6) | 14.3 | (12.9) | 2.6 | (4.2) | 200 | (181.7) |
| June | 86.2 | (59.9) | 11.6 | (12.6) | 2.0 | (1.8) | 133 | (132.1) |
| July | 129.8 | (63.4) | 10.8 | (10.7) | 2.7 | (1.3) | 137 | (154.8) |
| August | 109.8 | (65.0) | 12.1 | (10.8) | 1.9 | (2.6) | 234 | (235.1) |
| September | 41.0 | (44.4) | 14.3 | (15.6) | 5.8 | (4.3) | 338 | (360.1) |
| October | 22.4 | (49.2) | 16.7 | (17.1) | 5.6 | (6.3) | 567 | (517.6) |
| November | 11.4 | (51.4) | 18.3 | (19.6) | 8.9 | (7.9) | 686 | (630.3) |
| December | 76.6 | (52.3) | 20.8 | (19.5) | 10.7 | (10.1) | 701 | (701.5) |
| January | 46.4 | (49.3) | 22.0 | (24.4) | 12.5 | (11.5) | 725 | (686.2) |

# Chapter 4
# Full plot results

## 4.1 Introduction

Chapter 4 investigates results collected from quadrat cuts or data collected from whole plot assessments. All seed yield data are presented at 11% seed moisture content. Further detail on analysis methods is described within the respective sections. The parameters for all fitted figures are shown in Appendix B.

### 4.1.1 Lodging

Under certain environments and management lodging can occur in perennial ryegrass seed crops due to tillers lacking the strength to support the weight of developing seed heads and/or seed. Lodging is usually increased by the application of nitrogen fertiliser and adequate levels of soil moisture (Rolston *et al*. 2007b). Seed yield is often reduced when lodging occurs through either;

1. stems lying on the canopy surface shading those below, leading to reduced current photosynthate for seed filling and,

2. a reduction in the number of seeds produced

Lodging is common as stems approach anthesis near the end of tiller elongation. Elongation is a result of the intercalary meristem found above each node. Each internode on a tiller elongates independently and is promoted by gibberellic acid. Trinexapac ethyl (Moddus) is a plant growth regulator which is an acylcyclohexanedione inhibitor of the 3-β hydroxylation during gibberellic acid production (Rademacher, 2000). This leads to a shortening of internodes and thickening of stems leading to either reductions to overall lodging or a delay in lodging and results in an increase in the number of seeds produced per spikelet (Chastain *et al*. 2003, Rolston *et al*., 2007b). When Moddus is applied to perennial ryegrass in New Zealand, seed yield increases of 50% are common (Rolston *et al*., 2004). These results are similar to those achieved by Hebblethwaite *et al*., (1978) who recorded a 40% yield increase when lodging was prevented using wires (refer to section 2.3.7).

In New Zealand it is currently best practice to apply between 300 and 400 g TE/ha (1200 and 1600 ml/ha respectively) to perennial ryegrass seed crops with the aim of delaying lodging. Trinexapac ethyl activity is greatest when applied at Zadoks growth stage (GS) 32 (2 nodes for average of all tillers) (Zadoks 1974).

This experiment aimed to investigate the influence of sowing date, cultivar and Moddus plant growth regulator on anthesis date, seed yield, straw yield, harvest components, harvest index and seed germination.

## 4.2 Crop Development

Crop development stages were observed in all treatments on tagged main stem tillers (10 in each plot). Individual cultivars had different stem extension, ear emergence, anthesis and harvest dates. The stem extension and ear emergence dates were altered slightly by sowing date but differences reduced towards harvest (Table 4.1).

**Table 4.1.** Crop development on main stem tillers of three perennial ryegrass cultivars sown on two dates at Lincoln University, Canterbury in the 2008/09 season and treated with 800 ml/ha Moddus (a.i. 250 g/l Trinexapac ethyl).

| Growth/development stage | Cultivar and sowing date | | | | | |
|---|---|---|---|---|---|---|
| | Meridian | | Bronsyn | | Impact | |
| | 1/04/08 | 14/05/08 | 1/04/08 | 14/05/08 | 1/04/08 | 14/05/08 |
| Crop emergence | 17-Apr | 4-Jun | 17-Apr | 4-Jun | 17-Apr | 4-Jun |
| 2nd Node visible | 26-Sep | 15-Oct | 15-Oct | 23-Oct | 21-Oct | 28-Oct |
| Main stem head emergence | 12-Oct | 24-Oct | 29-Oct | 3-Nov | 19-Nov | 22-Nov |
| Main stem anthesis | 6-Nov | 15-Nov | 21-Nov | 23-Nov | 8-Dec | 13-Dec |
| 95% final seed weight (main stem) | 12-Dec | 13-Dec | 15-Dec | 15-Dec | 5-Jan | 5-Jan |
| Final plot harvest (mean 40% SMC*) | 23-Dec | 23-Dec | 27-Dec | 28-Dec | 15-Jan | 17-Jan |

## 4.3 Anthesis date

Date of peak anthesis for crops was affected by a sowing date by cultivar interaction (P<0.05). A later sowing date delayed anthesis of 'Meridian', by seven days compared with five for 'Grasslands Impact' and two for 'Bronsyn' between 1 April and 14 May sowing dates (Table 4.2). The progression of anthesis followed a normal distribution which was described using a Gaussian curve, (Figure 4.1) with no skewness detected. When cultivars and sowing dates were combined using peak anthesis date, as day zero, all cultivars displayed the same (P<0.05) level of anthesis both prior to and following the peak (Figure 4.1). Whole crop peak anthesis date was 6 ± 1.68 days later (P<0.05) compared with the main stems, due to the presence of $2^{nd}$ and lower order stems.

**Table 4.2.** Mean anthesis date (analyzed as Julian day) for whole plots of perennial ryegrass cultivars, 'Meridian', 'Bronsyn' and 'Grasslands Impact' sown on two dates at Lincoln University, Canterbury in the 2008/09 growing season.

| Cultivar | Mean anthesis dates | | |
|---|---|---|---|
| | Sowing date | | Cultivar mean |
| | 1st April 2008 | 14th May 2008 | |
| 'Meridian' | 15/11 | 22/11 | 18/11 |
| 'Bronsyn' | 26/11 | 28/11 | 27/11 |
| 'Grasslands Impact' | 12/12 | 17/12 | 15/12 |
| Sow date mean | 28/11 | 2/12 | |
| Effect | | P value | S.E.M |
| Sowing date*Cultivar | | 0.0452 | 0.8486 |
| Sowing date | | 0.0155 | 0.4091 |
| Cultivar | | 0.0000 | 0.6001 |

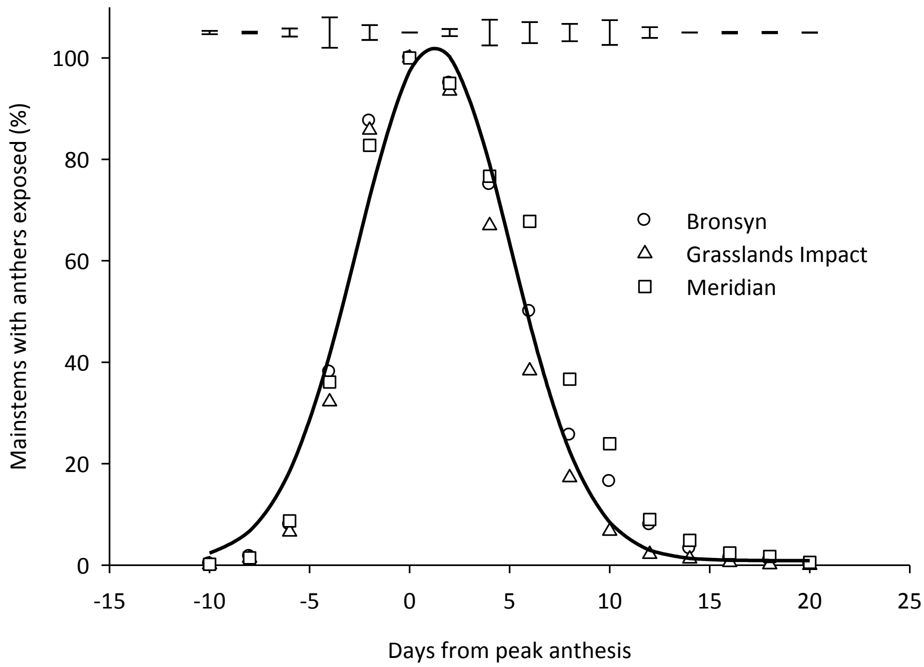



**Figure 4.1.** Percentage of stems of perennial ryegrass with florets open for pollination, average of two sowing dates (1 April and 14 May), three cultivars ('Meridian', 'Bronsyn' and 'Grasslands Impact') and three Trinexapac ethyl rates where peak anthesis date equals zero in the 2008/09 growing season at Lincoln University, Canterbury.

## 4.4 Lodging

Lodging occurred in all plots and was assessed each 4-6 days. A score of 0% meant the plot was fully standing, 50% meant the entire plot was leaning on a 45° angle while a 100% score indicates the entire plot was lying horizontal.

### 4.4.1 Analysis

Lodging was expressed against days after main stem head emergence (head emergence defined as 10 or more spikelets fully emerged).

Analysis included plotting lodging scores from individual treatment using MS Excel to visually identify the linear phase of 'change in lodging' for each plot. For the linear phase of lodging, linear regression (Equation 4.1) coefficients were generated using Genstat 14 for individual plots. Coefficients were analysed using analysis of variance for treatment differences. The location where the regression line crossed the x-axis was found by substituting zero for 'y' in Equation 4.2, thus gaining an estimate of the time of the onset of lodging (relative to mainstem ear emergence).

Equation 4.1 $Y = ax + c$

Where; a is the slope of the fitted line,

c is the intercept on the y-axis and,

x is any value on the x-axis.

Equation 4.2 $X = a - c / y$

Where; a is the slope of the fitted line,

c is the y-axis intercept and,

y is the value of interest on the y-axis.

### 4.4.2 Results

The absolute level of crop lodging (at harvest) was affected by Moddus ($P<0.01$; $F=112$) and a sowing date by cultivar interaction ($P<0.05$; $F=6.05$) but of none the Moddus interactions. The application of Moddus reduced crop lodging from 95 ± 2.87% (effectively horizontal) for 0 ml/ha Moddus to 56% (leaning on a 45° angle) for 800 ml/ha and 35% for 1600 ml/ha ($LSD_{0.05}$=8.4%). In the sowing date by cultivar interaction (Table 4.3), 'Bronsyn' (66 ± 2.87%) and 'Grasslands Impact' (74%) sown 1 April showed a higher level of lodging compared with sowing on 14 May ('Bronsyn' 47%, 'Grasslands Impact' 53%). Lodging for Meridian was the same (66%) for both sowing dates.

**Table 4.3.** Crop lodging % at harvest for three perennial ryegrass cultivars sown on two dates at Lincoln University, Canterbury in the 2008/09 growing season.

| | Sowing date | Cultivar | lodging %* | |
|---|---|---|---|---|
| | 1 April | 'Meridian' | 67 a | |
| | | 'Bronsyn' | 66 a | |
| | | 'Grasslands Impact' | 75 a | |
| | 14 May | 'Meridian' | 64 a | |
| | | 'Bronsyn' | 47 b | |
| | | 'Grasslands Impact' | 53 b | |
| Effect | P value | SEM | $LSD_{0.05}$ | |
| Moddus | <0.01 | 2.87 | 8.4 | |
| Sowing date*Cultivar | 0.025 | 2.93 | Within cultivar | 9.5 |
| | | | between cultivar | 13.0 |

* Note: 50% = crop leaning 45°, 100% = horizontal

### 4.4.3 Timing and rate of lodging

Cultivar and Moddus rate affected ($P<0.05$) the slope of the regression analysis for the linear phase of lodging (increase in lodging percentage/day). There were no interactions ($P<0.05$). Following the onset, lodging progressed quicker in 'Meridian' (2.12 ± 0.157%/day) compared with 'Bronsyn' (1.59%). 'Grasslands Impact' was similar (1.80%) to both 'Meridian' and 'Bronsyn' ($LSD_{0.05}$ = 0.362).

The change in lodging percentage/day was greater ($P<0.05$) where no Moddus was applied, 2.44 ± 0.118%/day compared with Moddus which was similar at 1.63%/day for 800 ml/ha and 1.44%/day at 1600 ml/ha.

The y-axis intercept was affected by sowing date, cultivar and Moddus rate, but none of their interactions. The sowing date and Moddus data are presented for individual cultivars in Figure 4.2

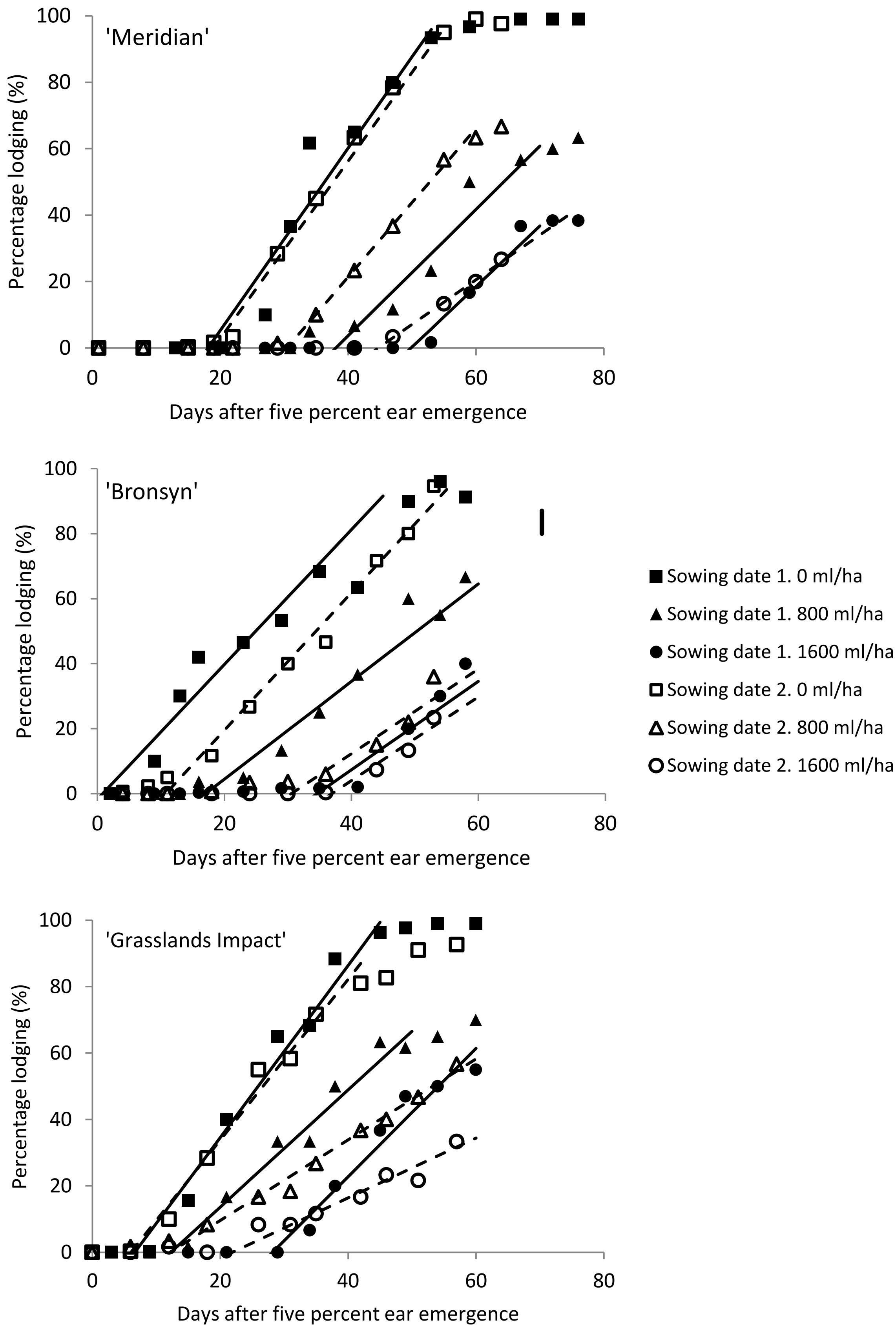



**Figure 4.2.** Lodging percentage (0 = fully standing, 50 = leaning 45°, 100 = horizontal) following five percent ear emergence for three cultivars of perennial ryegrass with two sowing dates (1 April, solid lines, and 14 May, dashed lines) and three application rates of Moddus (a.i. 250 g/l Trinexapac ethyl), Lincoln University, Canterbury, 2008/09 growing season. Error bar = SEM for sowing date*cultivar*Moddus, absolute value of lodging.

The onset of lodging (calculated x-axis intercept) included cultivar by Moddus (P<0.024) and sowing date by cultivar (P<0.035) interactions. For the cultivar by Moddus interaction, the addition of 800 ml/ha Moddus delayed lodging compared with the untreated for 'Meridian' and 'Bronsyn'. However, 1600 ml/ha was required to delay lodging in 'Grasslands Impact'. Within cultivars, 1600 ml/ha Moddus delayed lodging further than 800 ml/ha, but 800 ml/ha for 'Meridian' showed the same delay as 1600 ml/ha for 'Bronsyn', indicating individual cultivars responded differently to different rates of Moddus.

Sowing date had no effect on delaying the onset of lodging in 'Meridian' or 'Grasslands Impact' but in Bronsyn the second sowing date delayed the onset of lodging by 10 days ($LSD_{0.05}$ = 7.38). Across all sowing dates and cultivars, the application of Moddus delayed (P<0.05) lodging by 14.2 and 25.9 days for 800 and 1600 ml/ha respectively compared with the untreated plots (LSD =3.74).

## 4.5 Plot seed yield

### 4.5.1 Total seed produced

The total seed yield produced (those above 0.5 mg, achieved by air separation and weighing 200 individual seeds) was affected (P<0.05) by sowing date and Moddus but none of their interactions (Table 4.4). All data are presented for clarity in Table 4.5. Sowing on 14 May produced 2980 kg/ha of seed compared with sowing 2431 kg/ha from 1 April. The application of Moddus increased (P<0.05) yield from 2126 ± 65 kg/ha in the untreated control to 2774 kg/ha at 800 ml/ha and 3250 kg/ha at 1600 ml/ha ($LSD_{0.05}$ = 190). All cultivars produced the same weight of seed greater than 0.5 mg individual seed weight.

**Table 4.4** Total seed yield (kg/ha) (seed greater than 0.5 mg weight) of perennial ryegrass crops sown on two dates and treated with three 'Moddus' rates (a.i. 250 g/l Trinexapac ethyl) at Lincoln University, Canterbury, in the 2008/09 growing season.

| Moddus (ml/ha) | Sowing date | | | Moddus Mean |
|---|---|---|---|---|
| | 1 April | | 14 May | |
| 0 | 1881 | | 2371 | 2126 c |
| 800 | 2417 | | 3072 | 2744 b |
| 1600 | 2996 | | 3500 | 3248 a |
| Sowing date mean | 2431 b | | 2981 a | |
| Effect | P value | SEM | $LSD_{0.05}$ | |
| Sowing date | 0.0132 | 45.10 | 274 | |
| Moddus | 0.0000 | 65.35 | 190 | |

**Table 4.5** Total seed yield (kg/ha) (seed greater than 0.5 mg weight) of three cultivars of perennial ryegrass crops sown on two sowing dates and treated with three 'Moddus' rates (a.i. 250 g/l Trinexapac ethyl) at Lincoln University, Canterbury in the 2008/09 growing season.

| Sowing date | 1 April 2008 | | | Cultivar (kg/ha) | 14 May 2008 | | | Cultivar (kg/ha) | Cultivar mean (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| Cultivar | Moddus Rate (ml/ha) | | | | Moddus Rate (ml/ha) | | | | |
| | 0 | 800 | 1600 | | 0 | 800 | 1600 | | |
| 'Meridian' | 1611 | 2122 | 2933 | 2222 | 2360 | 3213 | 3476 | 3016 | 2619 |
| 'Bronsyn' | 1928 | 2595 | 3092 | 2538 | 2354 | 3168 | 3842 | 3121 | 2830 |
| 'Grasslands Impact' | 2105 | 2535 | 2963 | 2534 | 2398 | 2834 | 3184 | 2805 | 2670 |
| Moddus mean | 1881 | 2417 | 2996 | **2431 b** | 2371 | 3071 | 3501 | **2981 a** | |

| Effect | P value | SEM | $LSD_{0.05}$ |
|---|---|---|---|
| Sowing date | 0.0132 | 45.10 | 274.43 |
| Cultivar | 0.3060 | 93.68 | NS |
| Moddus | 0.0000 | 65.35 | 190.76 |

Note: bold figures give sowing date comparison.

### 4.5.2 First grade seed

Machine dressed seed yield (individual seed weight greater than 1.5 mg) was affected (P<0.05) by the main effect of sowing date, cultivar and Moddus rate but none of their interactions. However, all data are presented for clarity (Table 4.6). On average 550 kg/ha less seed was produced from sowing on 1 April compared with 14 May. Seed yield was higher from 'Bronsyn' than from 'Grassland Impact'. The Moddus rate also showed a 28% yield increase between 0 and 800 ml/ha and a further 25% increase at 1600 ml/ha (Table 4.6).

**Table 4.6.** First grade seed yield (kg/ha) (individual seed weight greater than 1.5 mg) of perennial ryegrass crops from three cultivars sown on two dates and treated with three Moddus rates (a.i. 250 g/l Trinexapac ethyl) at Lincoln University, Canterbury in the 2008/09 growing season.

| Sowing date | 1 April 2008 | | | Cultivar (kg/ha) | 14 May 2008 | | | Cultivar (kg/ha) | Cultivar mean (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| | Moddus Rate (ml/ha) | | | | Moddus Rate (ml/ha) | | | | |
| Cultivar | 0 | 800 | 1600 | | 0 | 800 | 1600 | | |
| 'Meridian' | 1324 | 1833 | 2474 | 1877 | 2059 | 2794 | 3056 | 2636 | 2257ab |
| 'Bronsyn' | 1487 | 2136 | 2665 | 2096 | 2152 | 2720 | 3383 | 2752 | 2424 a |
| 'Grasslands Impact' | 1536 | 1701 | 2279 | 1839 | 1734 | 1986 | 2476 | 2065 | 1952 b |
| Moddus mean | 1449 | 1890 | 2472 | **1937 b** | 1982 | 2500 | 2972 | **2485 a** | |
| Effect | | P value | SEM | LSD | | | | | |
| Sowing date | | 0.0021 | 17.68 | 107.60 | | | | | |
| Cultivar | | 0.025 | 97.23 | 317.10 | | | | | |
| Moddus | | 0.0000 | 67.05 | 195.72 | | | | | |

Note: bold figures indicate sowing date comparison.

### 4.5.3 Second grade seed

Second grade seed (seed between 0.5 and 1.5 mg) was affected ($P<0.05$) by cultivar and Moddus but none of their interactions. 'Grasslands Impact' produced 718 ± 39.8 kg/ha of second grade seed, more ($P<0.05$) than both 'Bronsyn' (406 kg/ha) and Meridian (362 kg/ha) which were similar ($LSD_{0.05}$ = 130). The application of Moddus at either 800 or 1600 ml/ha produced more ($P<0.05$) second grade seed compared with the untreated plots (Table 4.7).

**Table 4.7** Second grade seed yield (kg/ha) (seed of 0.5 -1.5 mg weight) of perennial ryegrass (Lolium perenne) as affected by two sowing dates, three cultivars and three Moddus (a.i. 250 g/l Trinexapac ethyl) rates at Lincoln University, Canterbury 2008/09 growing season.

| Sowing date | 1 April 2008 | | | Cultivar (kg/ha) | 14 May 2008 | | | Cultivar (kg/ha) | Cultivar mean (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| | Moddus Rate (ml/ha) | | | | Moddus Rate (ml/ha) | | | | |
| Cultivar | 0 | 800 | 1600 | | 0 | 800 | 1600 | | |
| 'Meridian' | 287 | 289 | 459 | 345 | 301 | 419 | 420 | 380 | **362b** |
| 'Bronsyn' | 441 | 459 | 427 | 442 | 202 | 448 | 459 | 370 | **406b** |
| 'Grasslands Impact' | 569 | 834 | 684 | 696 | 664 | 848 | 708 | 740 | **718a** |
| Moddus mean | 432 | 527 | 523 | 494 | 389 | 571 | 529 | 496 | |
| Effect | | P value | SEM | $LSD_{0.05}$ | | | | | |
| Sowing date | | 0.9611 | 29.46 | NS | | | | | |
| Cultivar | | 0.0004 | 39.80 | 129.8 | | | | | |
| Moddus | | 0.0146 | 33.01 | 96.34 | | | | | |

Note: bold figures give cultivar comparison.

### 4.5.4 Proportion of seed which is first grade seed

The proportion of seed converted into first grade seed was affected by the main plot effect of cultivar (P<0.05) but none of the interactions. Sowing date and Moddus had no effect (P<0.05). Both 'Meridian' and 'Bronsyn' converted ≈ 85% of total seed in to first grade seed compared with 73% for 'Grasslands Impact' ($LSD_{0.05}$ = 6.2%).

## 4.6 Yield components

### 4.6.1 Heads/m$^2$ and spikelets/head

Seed head production per m$^2$, the number of spikelets per seed head and the number of spikelets per m$^2$ were not affected by any of the main plot treatments or their interactions (Table 4.8).

**Table 4.8.** Components of yield, heads/m$^2$, spikelets/head and spikelets/m$^2$ for three perennial ryegrass cultivars and two sowing dates, Lincoln University, Canterbury, 2008/09 growing season.

| Yield component | 1$^{st}$ April 2008 | | | 1 April mean | 14$^{th}$ May 2008 | | | 14 May mean | Grand mean |
|---|---|---|---|---|---|---|---|---|---|
| | Meridian | Bronsyn | Impact | | Meridian | Bronsyn | Impact | | |
| Heads /m$^2$ | 2189 | 2200 | 2361 | 2225 | 2358 | 2299 | 2360 | 2398 | **2312** |
| Spikelets/ head | 21.3 | 22.3 | 21.6 | 21.7 | 20.3 | 19.8 | 21.9 | 20.3 | **21.0** |
| Spikelets/m$^2$ | 46649 | 49204 | 50937 | 48930 | 47916 | 45608 | 51641 | 48388 | **48659** |

| Heads/m$^2$ | P value | Spikelets/head | P value | Spikelets/m$^2$ | P value |
|---|---|---|---|---|---|
| Sowing date | 0.1350 | Sowing date | 0.1999 | Sowing date | 0.8062 |
| Cultivar | 0.1073 | Cultivar | 0.2218 | Cultivar | 0.1104 |
| Moddus rate | 0.9928 | Moddus rate | 0.0696 | Moddus rate | 0.3949 |

Note: key figures in bold.

### 4.6.2 Thousand Seed Weight

Thousand seed weight (TSW) was affected by the interaction of sowing date and cultivar but not Moddus (P<0.05). The sowing date and cultivar interaction was driven by the large difference in TSW between sowing dates for the cultivar 'Meridian', 0.28 g compared with 0.14 g for 'Grasslands Impact' and 0.11 g for 'Bronsyn' where the later sowing date had the higher TSW (Table 4.9). Approximately 50% of tillers for 'Meridian' sown on 1st April were affected by frost damage which may have influenced this result. Thousand seed weight from 14 May sowing was heavier (P<0.05) at

2.50 g compared with 2.32 from 1 April sowing. In all cultivars delayed planting resulted in higher TSW. Full interaction data for all yield components can be found in Appendix A.1.

**Table 4.9.** Thousand seed weight (g) of three cultivars of perennial ryegrass as affected by the treatments of sowing date, cultivar and Moddus rate at Lincoln University, Canterbury in the 2008/09 growing season.

| Cultivar | Sowing date | | | Cultivar mean |
|---|---|---|---|---|
| | 1st April 2008 | 14th May 2008 | | |
| 'Meridian' | 2.50 | 2.78 | | 2.64 |
| 'Bronsyn' | 2.51 | 2.62 | | 2.57 |
| 'Grasslands Impact' | 1.96 | 2.10 | | 2.03 |
| Sow date mean | 2.32 | 2.50 | | |
| Effect | P value | SEM | | $LSD_{0.05}$ |
| Sowing date | 0.0042 | 0.0115 | | 0.050 |
| Cultivar | 0.0000 | 0.0267 | | 0.062 |
| Sowing date*Cultivar | 0.0218 | 0.0378 | within sowing date | 0.087 |
| | | | between sowing date | 0.084 |

### 4.6.3 Seeds/spikelet

The number of seeds/spikelet greater than 1.5 mg was calculated from the first grade seed yield, TSW, head numbers and spikelets/head data (Table 4.10). The number of seeds per spikelet was affected by the interaction between sowing date and cultivar (P<0.028) and by Moddus (P<0.01) as a main effect. 'Meridian' and 'Bronsyn' produced fewer seeds/spikelet (1.66 ± 0.09) when sown on 1 April compared with 'Bronsyn' (2.34) when sown on 14 May ($LSD_{0.05}$ = 0.52). For Moddus, 0 ml/ha produced 1.5 ± 0.07 seeds/spikelet while the application of 800 ml/ha (1.8) and 1600 ml/ha (2.4) both increased (P<0.01) the number of seeds/spikelet ($LSD_{0.05}$ = 0.20).

**Table 4.10** Seeds/spikelet for two sowing dates, three Moddus rates (a.i. 250 g/l Trinexapac ethyl) and three perennial ryegrass cultivars grown at Lincoln University, Canterbury in the 2008/09 growing season.

| Cultivar | 1 April 2008 | | | cultivar mean | 14 May 2008 | | | cultivar mean |
|---|---|---|---|---|---|---|---|---|
| | 0 | 800 | 1600 | | 0 | 800 | 1600 | |
| 'Meridian' | 1.11 | 1.54 | 2.25 | 1.64 | 1.44 | 2.24 | 2.40 | 2.03 |
| 'Bronsyn' | 1.21 | 1.65 | 2.20 | 1.68 | 1.90 | 2.29 | 2.82 | 2.34 |
| 'Grasslands Impact' | 1.62 | 1.63 | 2.34 | 1.86 | 1.69 | 1.67 | 2.39 | 1.92 |
| Moddus mean | 1.31 | 1.61 | 2.26 | 1.73 | 1.68 | 2.07 | 2.54 | 2.09 |

| Effect | P value | SEM | | $LSD_{0.05}$ |
|---|---|---|---|---|
| Sowing date*Cultivar | 0.0278 | 0.088 | Within sow date | 0.288 |
| | | | Between sow date | 0.524 |
| Moddus rate | 0.0000 | 0.070 | | 0.205 |
| Sowing date | 0.0856 | 0.114 | | NS |
| Cultivar | 0.1747 | 0.062 | | NS |

### 4.6.4 Number of first grade of seeds/m$^2$

The number of first grade seeds per m$^2$ was affected by the main effects of sowing date ($P<0.05$) and Moddus ($P<0.01$) but none of their interactions. The application of Moddus increased the number of first grade seeds from ≈71,000 ± 3130 seed/m$^2$ for 0 ml/ha Moddus to ≈91,600 seed/m$^2$ for 800 ml/ha Moddus and up to 113,600 seed/m$^2$ for 1600 ml/ha Moddus. An average of approximately 21,000 seeds per m$^2$ were gained for the addition of each 800 ml/ha increment of Moddus (Table 4.11).

Sowing on 14 May (99,660 ± 1357 seeds/m$^2$) produced more seeds per m$^2$ compared with 1 April (84,480 seed/m$^2$) sowing ($LSD_{0.05}$ = 8,260).

**Table 4.11.** Number of first grade seeds per $m^2$ from perennial ryegrass when treated with three rates of Moddus (a.i. 250 g/l Trinexapac ethyl), grown at Lincoln University, Canterbury in the 2008/09 growing season.

| | Moddus rate (ml/ha) | Seeds/$m^2$ | Difference (seeds/$m^2$) |
|---|---|---|---|
| | 0 | 71026 c | |
| | | | 20571 |
| | 800 | 91597 b | |
| | | | 21996 |
| | 1600 | 113593 a | |
| Effect | P value | SEM | $LSD_{0.05}$ |
| Moddus | <0.001 | 3130 | 9136 |

### 4.6.5 Crop harvest index

Harvest index (HI) (assessed at final hand harvest, seed at 11% seed moisture content and stem as dry matter/ha) was influenced by an interaction (P=0.012) between cultivar and Moddus (Table 4.12) and the main effect of sowing date (P<0.01). The HI of both 'Meridian and 'Bronsyn' increased by ≈8% as Moddus rate increased from 0 – 1600 ml/ha, compared with a 2.5% increase for 'Grasslands Impact'. The HI increased as Moddus rate increased from both sowing dates by approximately 6% between the 0 and 1600 ml/ha Moddus treatments. Crops sown 14 May had a higher harvest index (18.9%) than those sown 1 April (14.6%) (Table 4.13). The late flowering cultivar, 'Grasslands Impact' had a lower harvest index than the earlier flowering cultivars. The application of Moddus increased harvest index from 13.5 ± 0.50% to 17.1 or 19.8% for 800 and 1600 ml/ha, respectively.

**Table 4.12.** Harvest index of three cultivars of perennial ryegrass crops sown on two dates and treated with and three Moddus rates (a.i. 250 g/l Trinexapac ethyl) in the 2008/09 growing season at Lincoln, Canterbury.

| Cultivar | Moddus rate (ml/ha) | | | | Cultivar mean |
|---|---|---|---|---|---|
| | 0 | 800 | | 1600 | |
| 'Meridian' | 13.8 | 17.4 | | 21.2 | 17.5 |
| 'Bronsyn' | 14.4 | 19.2 | | 23.4 | 19.0 |
| 'Grasslands Impact' | 12.3 | 14.7 | | 14.8 | 13.9 |
| Moddus mean | 13.5 | 17.1 | | 19.8 | |
| Effect | P value | SEM | | $LSD_{0.05}$ | |
| Cultivar*Moddus | 0.0122 | 0.8698 | Within cultivar | 2.54 | |
| | | | Between cultivar | 3.15 | |

**Table 4.13.** Harvest index of three cultivars of perennial ryegrass crops sown on two dates and treated with three Moddus rates (a.i. 250 g/l Trinexapac ethyl) in the 2008/09 growing season at Lincoln, Canterbury**.**

| Cultivar | 1st April 2008 Moddus Rate (ml/ha) | | | Cultivar mean (%) | 14th May 2008 Moddus Rate (ml/ha) | | | Cultivar mean (%) | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 800 | 1600 | | 0 | 800 | 1600 | | |
| 'Meridian' | 10.8 | 13.9 | 18.9 | 14.5 | 16.8 | 20.9 | 23.5 | 20.4 | 17.5 a |
| 'Bronsyn' | 12.2 | 16.7 | 21.2 | 16.7 | 16.6 | 21.7 | 25.6 | 21.3 | 19.0 a |
| 'Grasslands Impact' | 11.4 | 13.0 | 13.7 | 12.7 | 13.2 | 16.4 | 15.8 | 15.1 | 13.9 b |
| Moddus mean | 11.5 | 14.5 | 18.0 | **14.6 b** | 15.5 | 19.7 | 21.6 | **18.9 a** | 16.8 |

| Effect | P value | SEM | | $LSD_{0.05}$ |
|---|---|---|---|---|
| Sowing date | 0.0003 | 0.0515 | | 0.31 |
| Cultivar | 0.0033 | 0.7300 | | 2.38 |
| Moddus | 0.0000 | 0.5022 | | 1.47 |
| Cultivar*Moddus | 0.0122 | 0.8698 | Within cultivar | 2.54 |
| | | | Between cultivar | 3.15 |

Note; bold figures indicate sowing date differences.

### 4.6.6 Crop straw yield at harvest

Total non-seed dry matter (straw) at the final hand harvest was affected (P<0.001) by a cultivar by Moddus interaction and by the main effect treatment of sowing date. In both 'Meridian' and 'Bronsyn' the addition of Moddus did not alter the straw dry matter present at harvest. However in 'Grasslands Impact' the addition of Moddus at 1600 ml/ha increased (P<0.05) straw weight to 13,730 ± 406 kg DM/ha compared with 0 ml/ha Moddus (10,881 kg DM/ha) and 800 ml/ha (11,640 kg DM/ha) (Table 4.14). Sowing on 1 April produced more straw (11,338 ± 17 kg DM/ha) compared with 14 May (10,687 kg DM/ha). The late season cultivar, 'Grasslands Impact' produced more dry matter/ha compared with 'Meridian' and 'Bronsyn' which were similar (Table 4.14).

**Table 4.14.** Straw dry matter (less seed weight) present at harvest of perennial ryegrass (Lolium perenne) for two sowing dates, three cultivars and three Moddus rates (a.i. 250 g/l Trinexapac ethyl), 2008/09 growing season, Lincoln, Canterbury

| Cultivar | 1st April 2008 Moddus Rate (ml/ha) | | | Cultivar mean (kg DM/ha) | 14th May 2008 Moddus Rate (ml/ha) | | | Cultivar mean (kg DM/ha) | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 800 | 1600 | | 0 | 800 | 1600 | | |
| 'Meridian' | 11050 | 11600 | 10700 | 11117 | 10300 | 10600 | 10000 | 10300 | 10690 |
| 'Bronsyn' | 10750 | 10500 | 9900 | 10383 | 10800 | 9800 | 9800 | 10133 | 10690 |
| 'Grasslands Impact' | 11800 | 11450 | 14300 | 12517 | 11400 | 10350 | 13200 | 11650 | 12083 |
| Moddus mean | 11200 | 11183 | 11633 | **11339 b** | 10833 | 10250 | 11000 | **10700 a** | 11154 |

| Effect | P value | SEM | | $LSD_{0.05}$ |
|---|---|---|---|---|
| Sowing date | 0.0014 | 17 | | 104 |
| Cultivar | 0.0135 | 342 | | 1116 |
| Moddus | 0.2106 | 234 | | NS |
| Cultivar*Moddus | 0.0007 | 406 | Within cultivar | 1183 |
| | | | Between cultivar | 1474 |

Note; bold figures indicate sowing date differences.

### 4.6.7 Germination percentage of first grade seed

Germination of first grade seed was unaffected (P<0.05) by any of the treatments imposed (Table 4.15). All treatments achieved a final germination ranging between 93 and 98%. There was no difference or interactions (P<0.05) for dead or abnormal seeds/seedlings from any treatments.

**Table 4.15** Germination percentages for 18 different cultivar, sowing date and Moddus plant growth regulator (a.i. 250 g/l Trinexapac ethyl) combinations on perennial ryegrass grown for seed, Lincoln University, Canterbury 2008/09 growing season.

| Sowing Date | Cultivar | Moddus Rate (ml/ha) | Final germination |
|---|---|---|---|
| 1 April | 'Meridian' | 0 | 98 |
| | | 800 | 94 |
| | | 1600 | 94 |
| | 'Bronsyn' | 0 | 96 |
| | | 800 | 98 |
| | | 1600 | 97 |
| | 'Grasslands Impact' | 0 | 96 |
| | | 800 | 97 |
| | | 1600 | 93 |
| 14 May | 'Meridian' | 0 | 95 |
| | | 800 | 98 |
| | | 1600 | 97 |
| | 'Bronsyn' | 0 | 97 |
| | | 800 | 95 |
| | | 1600 | 96 |
| | 'Grasslands Impact' | 0 | 96 |
| | | 800 | 97 |
| | | 1600 | 95 |
| Average | | | 96 |

## 4.7 Discussion

The total seed yield produced was the same for all cultivars but increased when Moddus (Table 4.4) was applied and when sowing was delayed from 1 April to 14 May (Table 4.5). Individual seeds weighing greater than 0.5 mg were included in the total seed yield. These were considered to have endosperm deposits and be heavier than the empty lemma and palea. The conversion of total seed to first grade seed (that considered saleable and greater than 1.5 mg) was different between cultivars. Both 'Meridian' and 'Bronsyn' converted approximately 85% of seed produced to first grade compared with 'Grasslands Impact' at 73%. It is more common for authors to publish dressing losses compared with seeds converted to first grade seed. Dressing loss is commonly in the range of 2-10% of harvested seed yield which suggests many smaller seeds have already been removed in the threshing process. Some authors have investigated the conversion of seeds present at approximately mid seed fill into seeds of a saleable weight at harvest and found the percentage is generally less than 50% (Rolston *et al*., 2007, Trethewey and Rolston, 2009). 'Grasslands Impact' was the lowest yielding cultivar (1952 kg/ha), however not statistically different from 'Meridian' (2257 kg/ha) (Table 4.6). By definition, the conversion of total seed to first grade seed was through increased individual or thousand seed weight (TSW). The TSW for 'Grasslands Impact' (2.03 g) was lower than both 'Bronsyn' (2.57 g) and 'Meridian' (2.64 g), indicating a limitation to seed growth in 'Grasslands Impact' (Table 4.9). The TSW achieved in this experiment for 'Bronsyn' and 'Meridian' are considered high for diploid perennial ryegrass. Compared with 'Bronsyn', the TSW of 'Grasslands Impact' was 21% lower while the seed yield was 20% lower. 'Grasslands Impact' produced 77% more second grade seed, compared with 'Bronsyn' (Table 4.7). These results suggest the seed yield of 'Grasslands Impact' could be increased if assimilate was available and/or able to be transported to developing seeds.

Delaying sowing from 1 April to 14 May increased seed yield by 500 kg/ha for both total and first grade seed, the percentage conversion was unaffected. This result contradicts the data from Rolston and Archie (2005) where delayed sowing had a negative effect on harvested seed yield within individual experiments also sown at Lincoln. When two seasons of data were combined there was a 0.14% reduction in seed yield per hectare per day that sowing was delayed from 1 February. However, data presented from similar sowing dates as this experiment showed a neutral effect of sowing date in four cultivars where only extreme sowing dates were different from each other e.g. in 2004 sowing 22 February (1920 kg/ha) showed a higher ($P<0.05$) seed yield than all other sowing dates, whereas crops sown between 13 March – 30 May were similar (1618 kg/ha). In this experiment, delaying sowing date to 14 May reduced straw production by 640 kg/ha, concurrently seed weight was increased by 550 kg/ha and final crop HI was 4.3% higher (Table 4.13) suggesting greater allocation of assimilate towards the seed sink from the later sowing date. It is possible that

that a management or environmental factor influenced this result, but it is difficult to identify. There was a late season frost which may have affected 'Meridian' sown 1 April but 'Grasslands Impact' showed the same trend while reaching anthesis 30 days later. Therefore it is hypothesised that where the same number of potential seed sites are set, spring crop management and environmental conditions are more important than sowing date. As such further work is required to fully understand the role of sowing date in high producing seed crops where stem shorteners are applied.

There was no difference between cultivars in the number of first grade seeds produced/$m^2$. None of the treatments imposed had any effect on the number of seed heads/$m^2$ or the number of spikelets produced per head. Hence, the total number of spikelets/$m^2$ was the same in each crop (Table 4.8). Therefore, differences in harvested seed had to arise from either seed weight or the number of seeds/spikelet (Table 4.10). Moddus increased seed yield by 28% for the first 800 ml/ha applied, followed by an additional 25% when a further 800 ml/ha was applied. Moddus had no effect on TSW or the number of spikelets/$m^2$, therefore an increase in the number of first grade seeds/$m^2$ describes the seed yield increase from Moddus (Table 4.11). The increase in seeds/$m^2$ was a result of more seeds/spikelet reaching 1.5 mg (Table 4.10). The change in seeds/spikelet was also shown by Chastain *et al*., (2003). Borm & van den Berg (2008) have shown that Moddus may increase the number of seed head bearing tillers under certain situations but did not ascertain what those conditions were. These results describe the changes in yield location but do not explain why the number of seeds produced per spikelet changed, which will be explored in Chapter 5.

In most cases treatment effects which produced greater residue dry matter at harvest had a lower percentage of dry matter as seed and therefore a lower HI. The grand mean for dry matter/ha was 11,100 kg DM/ha (Table 4.14) while the mean HI was 16.8% (Table 4.13). In this experiment HI was influenced by increasing seed yield while DM at harvest remained similar. Delayed sowing reduced total DM production but increased seed yield, hence HI was greater. In most cases, the application of Moddus increased harvest index (13.5 – 20%, Table 4.12) due to increasing seed yield while DM/ha remained constant. However, for 'Grasslands Impact' the residue DM/ha at harvest increased when 800 and 1600 ml/ha of Moddus were applied. This gave similar levels of HI even though seed yield increased. Reasons for the increase in DM when Moddus was applied to 'Grasslands Impact' are unclear but may include greater utilisation of incoming radiation due to reduced lodging (Griffith 2000). Borm & van den Berg (2008) showed that dry matter production was increased by the application of Moddus when applied later in the season at approximately Zadoks growth stage 33-37 (3 three nodes visible and tip of final leaf emerging). However, when applied at a single application timing caused no difference in dry matter production, a result supported by Silberstein *et al*. (2003).

The overall range of harvest index (13.5 - 20%) shown in this experiment was low compared with annual cereal crops (Hay, 1995). Based on the genetic improvements of cereals (Austin *et al*., 1980), the HI of perennial ryegrass seed crops could be improved through plant breeding and crop management. However, a limitation to ryegrass seed crops compared with annual cereals is the number of tillers which are produced and the potential seed production from these. Generally perennial ryegrass cultivars are selected for tillering and dry matter production and as such produce many more tillers than annual cereals (Langer, 1980). Lee (1991) demonstrated that the order of tiller development influenced the final grain weight produced per stem and on an individual grain basis in barley. Similar results were shown for perennial ryegrass by Colvill and Marshal (1984) where older tillers produced higher seed yields. Reddy (1992) showed that primary tillers produced 197 seeds per panicle compared with 177 and 97 for later emerged, smaller tillers in Phalaris (*Phalaris aquatic* L.). Therefore the potential to improve HI may be reduced by the number of second, third and four order tillers which produce seed heads.

The main effects of cultivar, sowing date and Moddus were all implicated in the absolute lodging percentage at harvest (Table 4.3) and the onset of when lodging began (Figure 4.2). Lodging progressed more quickly in 'Meridian' than 'Bronsyn' and 'Grasslands Impact'. The cultivar differences suggest variation between cultivars in their ability to resist lodging (stem strength). Stem strength is often referred to in cereal production (Hay and Walker, 1989) but never reported in perennial ryegrass. For perennial ryegrass where increased stem strength may result from increased lignified tissue or secondary cell wall thickening (Wilson, 1990) may reduce palatability of forage for livestock (Stone, 1994). Delaying the sowing date from 1 April to 14 May delayed lodging in 'Bronsyn' but not in 'Meridian' or 'Grasslands Impact', the reasons for this are not immediately clear. In cereals delayed sowing generally reduces the risk of lodging through a reduction in dry matter production (Hay and Walker, 1989). In this experiment dry matter production was different ($P<0.05$) between sowing dates, however 600 kg/ha is insufficient to cause the difference in lodging when 11000 kg DM/ha is present (5%). The difference did not alter the onset of lodging in 'Meridian' or 'Grasslands Impact'.

The application of Moddus reduced overall lodging, delayed the onset of lodging and slowed the progression of lodging after the onset occurred. This result is similar to those presented by Silberstein *et al*. (2003), Borm & van den Berg (2008) and Rolston *et al*. (2010). Lodging has been reported to reduce seed yield through reduced assimilate supply to developing seeds (Clemence and Hebblethwaite, 1984), reduced pollination (Wright and Hebblethwaite, 1979) and low seed set (Burbidge *et al*., 1978), presumably when lodging occurs prior to anthesis. When lodging occurs the canopy losses the ability to capture and utilise incoming solar radiation, thereby reducing total photosynthesis. Concurrently when lodging occurs respiration occurs at the same rate leading to less assimilate available for seed filling (lower yield). Lodging during December and January occurs during the period when incoming PAR receipts are near maximum, leading to greater wastage compared with other periods of the year.

The progression of cultivars through developmental stages occurred in the expected sequence for both seed head emergence and anthesis date (Table 4.1, Table 4.2) i.e. 'Meridian', 'Bronsyn' and 'Grasslands Impact' (Agriseeds 2006). All cultivars displayed the same pattern of anthesis where the majority of stems completed anthesis within a 15-18 day timeframe (Figure 4.1), however the spread lasted 30 days. This result is similar to that of Warringa *et al*. (1998) who showed that it took 15 days for all florets to flower on an individual stem of perennial ryegrass.

The winter of 2008 was wet with waterlogging and flooding experienced across the experimental site on numerous occasions during July and August. However, with the exception of limited plant loss the winter weather conditions are not expected to have influenced these results. Perennial ryegrass has the ability to adjust harvest components to suit the environmental conditions e.g. tillering allows for the loss of neighbouring plants. The final frost of the 2008/09 growing season occurred during head emergence for the 1 April sowing of Meridian. The frost destroyed the top half of approximately 25% of seed heads, however while visually disastrous the achieved seed yields appear in line with similar treatments (Photo 2). Within yield components there is an ability to compensate if one component is reduced in frequency (yield component compensation). Yield components are often negatively related among themselves, for example; an increase in seed number often leads to reduced grain size, or as the case in this example, reduced spikelet number lead to an increased number of seeds/spikelet. This demonstrates the 'plastic' nature of yield components in species such as perennial ryegrass. Due to the developmental stage when the frost occurred, fertile spikelets were removed and there was no effect on germination (Table 4.15).

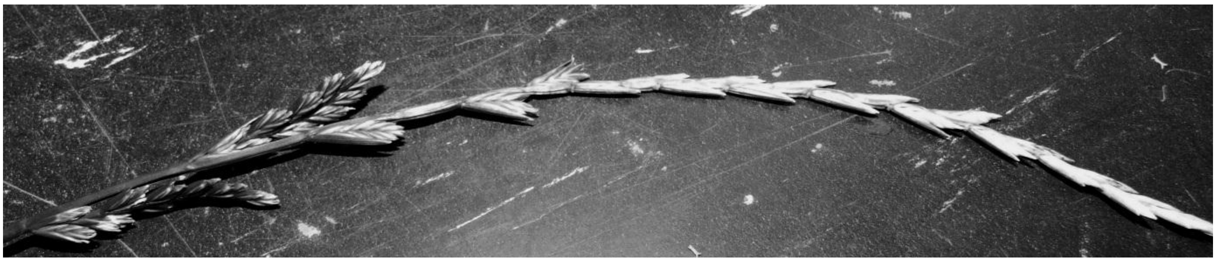


**Plate 2.** Compensation by lower spikelets when the central through distal spikelets were destroyed by frost at ear emergence in 'meridian' when sown on 1 April at Lincoln University during the 2008/09 growing season.

Chapter 4 has identified that seed yield was increased as Moddus rate increased through the production of a greater number of seeds/$m^2$ primarily due to more seeds/spikelet. Chapter 5 attempts to identify some possible mechanisms why Moddus promotes a greater number of seeds/spikelet while quantifying the parameters required to develop a simulation model for seed growth in perennial ryegrass.

# Chapter 5

# Seed growth on individual stems of perennial ryegrass

## 5.1 Introduction

Chapter 5 deals with individual main stem data collected at three to four day intervals as described in Section 3. Chapter 5 contains background information for sections on seed growth, change in stem weight and harvest index. The methods used to fit curves and generate calculated sets of data are included were appropriate. This chapter investigates changes in seed and stem dry weight following anthesis. All seed and stem weights in chapter 5 are presented on a dry weight basis. The parameters for all fitted figures are shown in Appendix B.

**Seed growth**

Seed or grain growth follows a sigmoidal growth curve from anthesis to maturity (Hay and Walker, 1989). This gives three recognised phases which have been well documented for wheat (Loss *et al*., 1989). Phase one follows anthesis and involves a short lag phase which includes pollination. There is little or no increase in seed dry weight during this phase (Loss *et al*., 1989). Phase two is characterised by a near linear increase in seed dry weight to a maximum at physiological maturity. Phase three, or seed maturation involves a dry down period. In perennial ryegrass, maximum dry weight and dry down phase coincides with the onset of seed shedding and in seed crop practices, time of swathing for harvest.

During the linear phase of growth, seed weight increase is a function of time (Gallagher, 1976; Brocklehurst, 1977). Therefore final seed weight can be quantified by differences in the rate or duration of the linear phase of growth. Seed growth can be analysed in relation to either calendar days or thermal time. Calendar days have been used to describe the diurnal cycle, assimilate accumulation and respiration in seed growth (Savin and Nicholas, 1996). Sofield *et al*. (1977) used analysis by days to interpret grain growth characteristics in wheat. However, the grain filling process is more aligned with environmental factors of which temperature is often given highest priority (Slafer and Savin, 1991). Therefore seed growth as described by thermal time (°C days), may be a stronger predictor of phase duration in ryegrass than calendar days. Analyses based on temperature accumulation allows for the quantification of differences in temperature variation associated with variation in both sowing date and location (Russelle *et al*., 1984) and thus could be used for analysis in this chapter.

**Change in stem weight**

The increase in seed yield following anthesis depends on the translocation of assimilate, mainly fructans and sucrose, from source organs such as leaves, stem or other photosynthetic tissue to the seed (Griffith, 2000). An organ is defined as a source or sink depending on the direction of net assimilate transport, either from or to it respectively. In cereals, the developing grain becomes the major sink for assimilate following anthesis but in perennial species new leaves, roots and the mature stem can also act as a sink. When there is more than one sink it is the relative strength of each sink to attract assimilates that is important.

Stored and remobilised carbohydrate can be estimated by changes in stem dry weight following anthesis. The contribution of stem assimilates to final yield has been reported to range from 10 -25% in wheat and up to 80% in barley (Borrell *et al*., 1989). The transport of assimilate from vegetative tissue to sinks is through the phloem. Therefore the capacity of the phloem, a function of cross section area and the number of vessels leading to the sink, may be an important factor in limiting the transport of assimilate.

Following anthesis, increasing stem dry weight indicates sugar being stored within the stem while a decrease in dry weight represents either respiration losses or transport of assimilate to a sink outside the stem. If seed filling is source limited then stem weight should reduce to a minimum as assimilate is remobilised. In contrast, if it is sink limited then unused assimilate would accumulate and stems increase in weight. Sink limitations can be either a physical restriction to assimilate flow or due to limited storage space.

This chapter aims to describe seed growth and changes in stem weight relative to thermal time and to investigate if seed production in perennial ryegrass is source or sink limited.

## 5.2 Seed Growth in Perennial Ryegrass

### 5.2.1 Curve fitting

To describe the pattern of seed growth a series of curves were fitted using Genstat 14. Logistic, generalised logistic and Gompertz growth curves were fitted. As a result of seed shedding many plots showed a decline in seed dry weight of the final data points, post physiological maturity. Therefore data points where seed shedding was recorded were removed and replaced with the last recorded weight to enable realistic curve fitting. A few individual plots required additional data points beyond the final dry weight to provide symmetry to enable a sigmoidal growth curve to be fitted. For each plot, the curve of best fit was described as that which had an $R^2$ value greater than or similar to the other curves and fitted the actual data points with a low standard error for the predicted parameters.

In each case a logistic curve was fitted first, followed by a generalised logistic and/or Gompertz growth curves. When reviewing curves if the logistic curve had a standard error >10% then the generalised logistic or Gompertz curves were assessed. The curve which then described the data most accurately, for the least complexity, was chosen. In most cases the generalised logistic did not fit the data without errors. If all lines produced a poor fit e.g. $R^2$ approx. 80 then the line of least complexity was used which is the logistic.

The logistic curve is a symmetrical sigmoidal growth curve (Equation 5.1) with four parameters; 'A', defined as the curve point (0 mg on the y-axis) of the curve when the x-axis equals zero. 'C' is the upper maximum that defines maximum final seed weight. 'B' is the mean slope over the entire curve and 'M' is the point of inflection of the curve on the x-axis which represents time to 50% final weight. X is any point on the x-axis.

Equation 5.1 $$Y = A + C / (1 + \mathit{exp}( - B (X – M)))$$

The generalised logistic curve (Equation 5.2) fits a modified symmetrical sigmoidal growth curve. It includes the parameter , 'T' which is a power parameter allowing the indication of an arrested exponential (i.e. growth continues exponentially and then stops) if T>10. All other parameters remain the same as the logistic curve.

Equation 5.2 $$Y = A + C / 1 + T\, exp( - B ( X – M )))^{*1/T}$$

The Gompertz curve (Equation 5.3) fits an asymmetricl growth curve about the inflection point.

Equation 5.3 $$Y = A + C\, exp( - exp( - B ( X – M )))$$

From the curve parameters, the duration of the linear phase of seed growth was calculated, defined as the interval between 5 and 95% of final seed weight (Gallagher *et al*., 1976). Final seed weight is given by parameter 'C' in the curve fitting equations. The duration of linear growth is calculated by using Equation 5.4 to establish x axis values for 5 and 95% of final seed weight (days and °C days). This is achieved through substituting the values of 5% and 95% of final seed weight for 'Y' in Equation 5.4. The duration of the linear phase (days and °C days) is established by subtracting the value of 5% final seed weight (x axis value) from the 95% value generated. The duration of the lag phase is estimated as the time from anthesis to 5% of the maximum seed weight. The mean slope over the linear phase is calculated by dividing the weight increase from 5% to 95% of final seed weight by the time taken for the same period in days or °C days (i.e. the duration of the linear phase).

Equation 5.4 $$X = (M – 1/B)LN((A+C/Y) – 1)$$

### 5.2.2 Statistical analysis.

All values were calculated for individual plots. Differences in the parameters, B, C, M and the derived values of mean seed filling rate, duration of the lag phase, time to 95% final weight, duration of the linear phase and slope of the linear phase, were compared using analysis of variance (ANOVA) between replicates, sowing date, cultivar and plant growth regulator treatments. Means separation was based on least significant difference (LSD) tests ($\alpha = 0.05$). Analysis of variance and LSD testing were carried out using Genstat 14.

Base temperature (Tb) was determined from observed seed weight regressed against thermal time (Tt). The $R^2$ values remained constant as Tb increased from 0 to 8 °C (91.1 vs. 91.3 respectively). Such increase is minor and has little practical significance. The ANOVA for the $R^2$ values showed no difference (P=998.0). Therefore, a Tb of zero was chosen for Tt discussions of seed growth because it allows comparison of all treatments (Moot *et al*., 2000).

## 5.3 Results

### 5.3.1 Main stem anthesis

Date of peak anthesis on main stems was affected by a sowing date by cultivar interaction (P<0.05) and the main effect of Moddus. Sowing date had the greatest influence on 'Meridian' (Figure 5.1), delaying anthesis by 11 days compared with five and two days for 'Grasslands Impact' and 'Bronsyn' respectively for 1 April and 14 May sowing dates (Table 5.1). Main stem peak anthesis date was 6 ± 1.68 days earlier (P<0.05) than the whole plots (section 4.1) where a range of tiller ages were present.

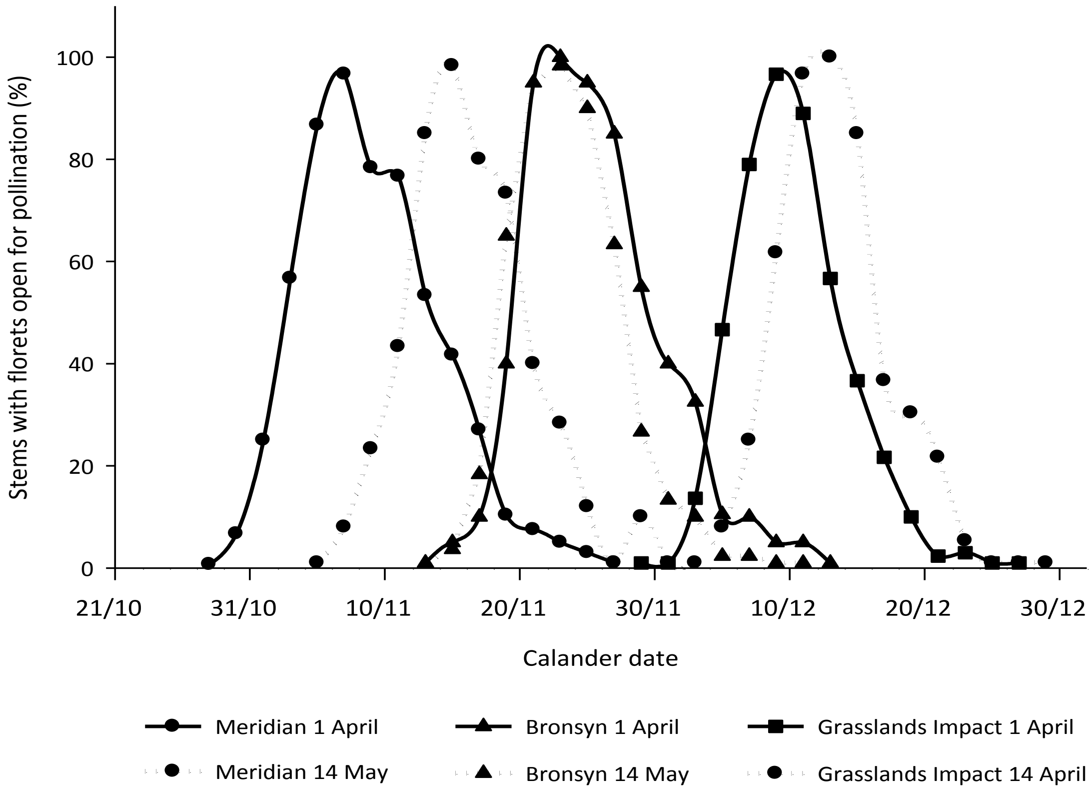



**Figure 5.1.** Percentage of stems with florets open for anthesis for three perennial ryegrass cultivars, (mean of three Moddus (a.i. 250 g/l Trinexapac ethyl) rates) sown on 1 April (solid lines) or 14 May (dashed lines) at Lincoln University, Canterbury in the 2008/09 growing season

**Table 5.1.** Mean anthesis date for main stems of perennial ryegrass cultivars, 'Meridian', 'Bronysn' and 'Grasslands Impact' from two sowing dates at Lincoln University, Canterbury in the 2008/09 growing season.

| Cultivar | Peak anthesis dates | | |
|---|---|---|---|
| | Sowing date | | Cultivar mean |
| | 1 April 2008 | 14 May 2008 | |
| 'Meridian' | 6/11 | 15/11 | 10/11 |
| 'Bronsyn' | 21/11 | 23/11 | 22/11 |
| 'Grasslands Impact' | 8/12 | 13/12 | 11/12 |
| Sow date mean | 22/11 | 27/11 | |
| Effect | Sig | P value | S.E.M |
| Sowing date*Cultivar | * | 0.034 | 0.948 |
| Sowing date | * | 0.026 | 0.868 |
| Cultivar | *** | 0.000 | 0.948 |

Anthesis was delayed by one day when Moddus was applied at 800 and 1600 ml/ha (P<0.05) (Table 5.2). There was no Moddus by cultivar interaction.

**Table 5.2.** Mean anthesis date of three perennial ryegrass cultivars ('Meridian', 'Bronsyn' and 'Grasslands Impact') following the application of three rates of Moddus (a.i. 250 g/l Trinexapac ethyl) at Lincoln University, Canterbury, in the 2008/09 growing season.

| Sowing date | Mean anthesis date (calendar date 2008) | | | |
|---|---|---|---|---|
| | Moddus application rate (ml/ha) | | | |
| | 0 | 800 | | 1600 |
| 1 April 2008 | 22/11 | 21/11 | | 22/11 |
| 14 April 2008 | 26/11 | 28/11 | | 28/11 |
| Moddus mean | 24/11a | 25/11b | | 25/11b |
| Effect | Sig | P value | SEM | $LSD_{0.05}$ |
| Sowing date*Moddus | NS | 0.0813 | 0.725 | NS |
| Moddus | * | 0.0251 | 0.3341 | 0.97 |

## 5.4 Rachis dry weight.

Cultivar was the only treatment to influence (P<0.05) rachis dry weight at both anthesis and final harvest. At anthesis, the rachis of 'Grasslands Impact' was lighter (44.3 mg) than that of 'Meridian' (53.8 mg) while 'Bronsyn' was similar to both cultivars (50.4 mg) (Table 5.3). At final harvest the rachis of 'Grassland Impact' was lighter (44.3 mg) than 'Meridian' and 'Bronsyn' which were similar

(57 mg). There was no change (P<0.05) in rachis dry weight between anthesis and final harvest for any treatments.

**Table 5.3.** Rachis dry weight at anthesis and final harvest for three cultivars of perennial ryegrass grown at Lincoln University, Canterbury in the 2008/09 growing season**.**

| Cultivar | Rachis dry weight (mg) | | Mean |
|---|---|---|---|
| | Anthesis | Harvest | |
| 'Meridian' | 53.8 a | 57.8 a | 55.80 |
| 'Bronsyn' | 50.4 ab | 56.3 a | 53.35 |
| 'Grasslands Impact' | 44.3 b | 43.8 b | 44.05 |
| Mean | 49.5 | 52.6 | |
| P value | 0.047 | 0.009 | |
| S.E.M | 2.26 | 2.52 | |
| $LSD_{0.05}$ | 7.37 | 8.26 | |
| Between assessment comparison | | P = 0.106 | SEM = 1.37 |

## 5.5 Changes in seed dry weight

Seed growth (dry weight/head, mg) analysis was examined as a function of either days after peak anthesis (DAPA) or thermal time (°C days) after peak anthesis. Seed growth is assumed to have started at anthesis, therefore 'A', the curve origin was 'set' to zero, thus 'A' was excluded from analysis of variance.

### 5.5.1 Days after peak anthesis

For all individual plots the logistic curve best described seed growth. Sowing date had no effect (M, P<0.141; C, P<0.149; B, P<0.181) on curve parameters (Table 5.4). In contrast cultivar affected (P<0.05) 'M', 'C' and 'B' and Moddus affected 'C' (P<0.05), but there were no treatment interactions.

**Table 5.4.** P values and treatment means generated through analysis of variance (Genstat 12) for logistic curve parameters of seed dry weight expressed as days after mean anthesis; 'M' (inflection point), 'C' (upper maximum) and 'B' (mean curve slope) during the seed growth phase in three cultivars of perennial ryegrass (*Lolium perenne* L.), Lincoln University, 2008/09 season.

| Treatment | ANOVA P value and Curve parameters | | |
|---|---|---|---|
| | M | C | B |
| Sow date | 0.141 | 0.149 | 0.181 |
| Cultivar | 0.002 | 0.018 | 0.044 |
| Moddus | 0.29 | 0.001 | 0.723 |
| 'Meridian' | 26.52 | 193 | 0.183 |
| 'Bronsyn' | 19.47 | 162 | 0.298 |
| 'Grasslands Impact' | 20.45 | 150 | 0.268 |
| Grand Mean | 22.14 | 168.3 | 0.25 |
| S.E.M | 0.956 | 8.45 | 0.0273 |
| $LSD_{0.05}$ | 3.117 | 28.15 | 0.0891 |

Individual figures were drawn (Figure 5.2) for each cultivar where Moddus rate was expressed as individual lines within each cultivar. 'C' was fitted for values of the Moddus by cultivar interaction (P=0.378) due to both cultivar and Moddus influencing curve parameters (Table 5.5). For Moddus, 'C' for 0 ml/ha (136 ± 10.8 mg) was different to 800 and 1600 ml/ha which were similar at 174 and 197 mg ($LSD_{0.05}$ = 31.5), respectively. For cultivar, 'Bronsyn' (166 ± 8.54 mg) was similar to both 'Grasslands Impact' (149 mg) and 'Meridian' (193 mg). The point of inflection ('M', days to 50% final seed weight) was later (P<0.05) for 'Meridian' at 26.5 ± 0.95 days compared with 'Bronsyn' (19.5) and 'Grasslands Impact' (20.45). The average curve slope ('B') for 'Grasslands Impact' (0.268 ± 0.027) was similar to 'Meridian' (0.183) and 'Bronsyn' (0.298) but these two differed from each other ($LSD_{0.05}$ = 0.0981).

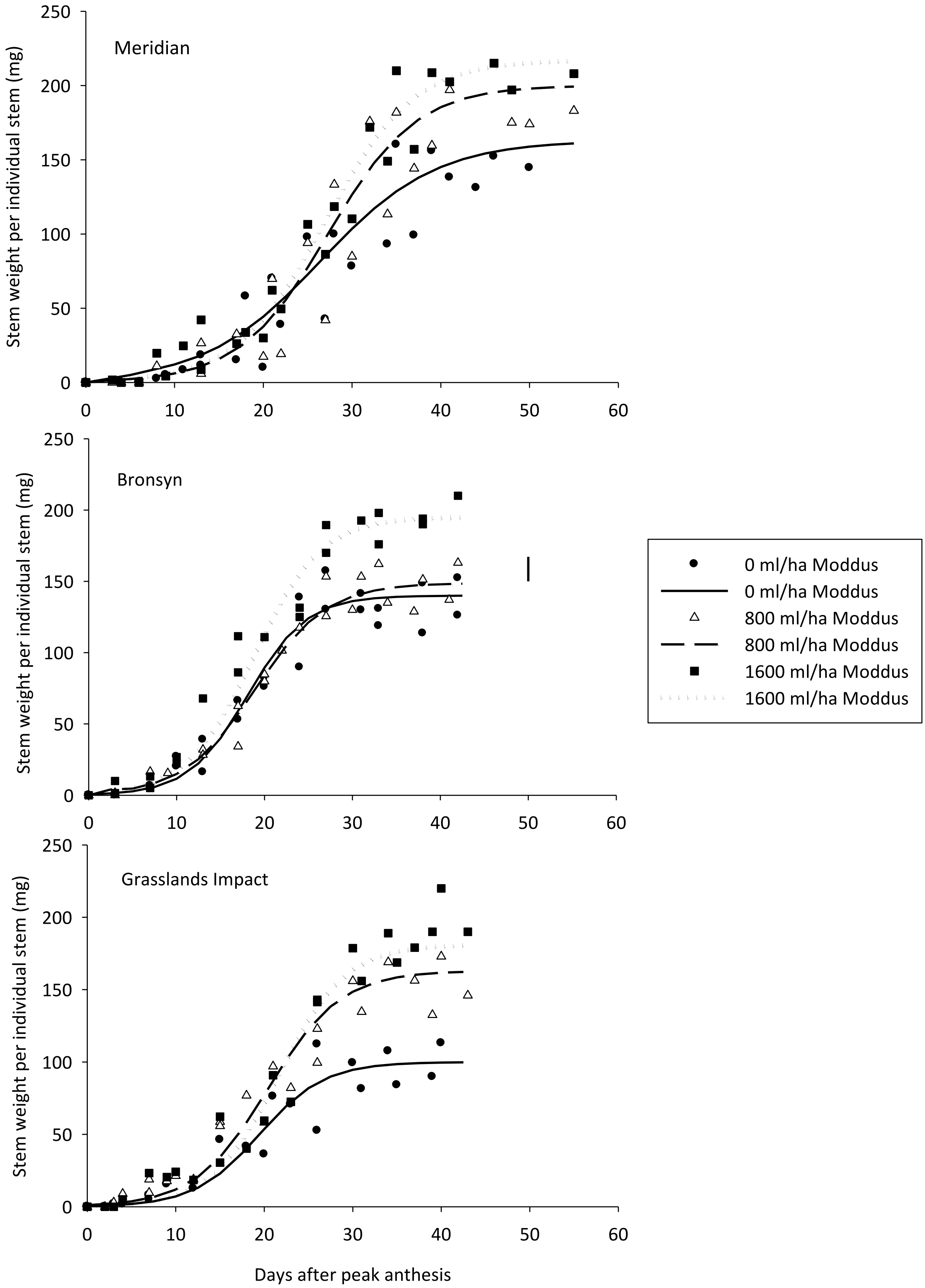



**Figure 5.2.** Seed growth expressed as a function of time (days) after peak anthesis for three rates of Moddus (a.i. 250 g/l Trinexapac ethyl) applied to three cultivars of perennial ryegrass, mean of two sowing dates (SD1 =1 April 2008, SD2 =14 May 2008). Error bar = S.E.M for Cultivar*Moddus at final harvest.

**Table 5.5.** Maximum curve values ('C') for seed growth as influenced by the plant growth regulator, Moddus (250 g/l Trinexapac ethyl), for three perennial ryegrass cultivars grown at Lincoln University during the 2008/09 growing season.

| Cultivar | Moddus rate (ml/ha) | | |
|---|---|---|---|
| | 0 | 800 | 1600 |
| 'Meridian' | 163 | 212 | 205 |
| 'Bronsyn' | 143 | 147 | 208 |
| 'Grasslands Impact' | 105 | 163 | 178 |
| Mean | 136 | 174 | 197 |
| | Moddus | Cultivar*Moddus | |
| P value | 0.002 | 0.378 | |
| S.E.M | 10.8 | 17.5 | |
| $LSD_{0.05}$ | 31.5 | NS | |

There was no sowing date, cultivar or Moddus effect (P<0.05) on the lag phase (time to 5% of final dry weight) at 11.2 ± 0.95 days. There was also no effect (P<0.05) on the duration of the linear phase (16.32 ± 1.3 days). There was a trend (P<0.056) for the slope of the linear phase to increase with Moddus rate (Table 5.6). Moddus was a sub sub plot treatment and therefore only tested against two degrees of freedom.

**Table 5.6.** Slope of the linear phase of seed growth in perennial ryegrass for three rates of Moddus plant growth regulator. Average of three cultivars and two sowing dates at Lincoln University, Canterbury in the 2008/09 growing season.

| Moddus rate (ml/ha) | Slope of the linear phase (mg/head/day) |
|---|---|
| 0 | 9.64 |
| 800 | 10.84 |
| 1600 | 12.85 |
| P value | 0.056 |
| S.E.M | 0.57 |

‘Meridian’ took longer (P<0.01) to reach 95% of final seed weight at 32.5 ± 1.28 days compared with ‘Bronsyn’ and ‘Grasslands Impact’ which were similar at 25.5 days ($LSD_{0.05}$ = 4.17)(Table 5.7).

**Table 5.7.** Time (days) to reach 95% of final seed weight following anthesis for three cultivars of perennial ryegrass. Average of three cultivars and two sowing dates at Lincoln University, Canterbury in the 2008/09 growing season.

| Cultivar | Time to 95% seed fill (days) |
|---|---|
| ‘Meridian’ | 32.5 |
| ‘Bronsyn’ | 24.5 |
| ‘Grasslands Impact’ | 25.5 |
| P value | 0.004 |
| S.E.M | 1.28 |
| $LSD_{0.05}$ | 4.17 |

## 5.5.2 Thermal time

Seed growth as a function of accumulated thermal time was described by the Logistic model (Figure 5.3). Sowing date had no effect (P<0.05) on curve parameters (Table 5.8).

Cultivar affected (P<0.05) the mean slope (‘B’) with a trend (P<0.074) to influence the maximum weight (‘C’). By comparison Moddus influenced ‘C’ (P<0.001) but there were no interactions. For ‘Grasslands Impact’ the mean curve slope (‘B’) was similar (0.016 ± 0.001) to both ‘Meridian’ (0.012) and ‘Bronsyn’ (0.018) but they were different to each other (LSD0.05 = 0.00198).

**Table 5.8.** P values and treatment means generated through analysis of variance (Genstat 12) for logistic curve parameters when expressed in thermal time, base temperature = 0°C; ‘M’ (inflection point), ‘C’ (upper maximum) and ‘B’ (mean curve slope) during the seed growth phase for three cultivars of perennial ryegrass grown at Lincoln University, in the 2008/09 growing season.

| Treatment | ANOVA P value | | |
|---|---|---|---|
| | M | C | B |
| Sow date | 0.822 | 0.082 | 0.315 |
| Cultivar | 0.224 | 0.052 | 0.045 |
| Moddus | 0.226 | 0.001 | 0.147 |
| Cultivar | Curve parameter values | | |
| | M | C | B |
| ‘Meridian’ | 314 | 192 | 0.012 |
| ‘Bronsyn’ | 279 | 172 | 0.018 |
| ‘Grasslands Impact’ | 294 | 151 | 0.015 |
| Grand Mean | 295.6 | 171.48 | 0.015 |
| S.E.M | 13.22 | 9.75 | 0.001 |
| $LSD_{0.05}$ | 43.1 | 31.8 | 0.005 |

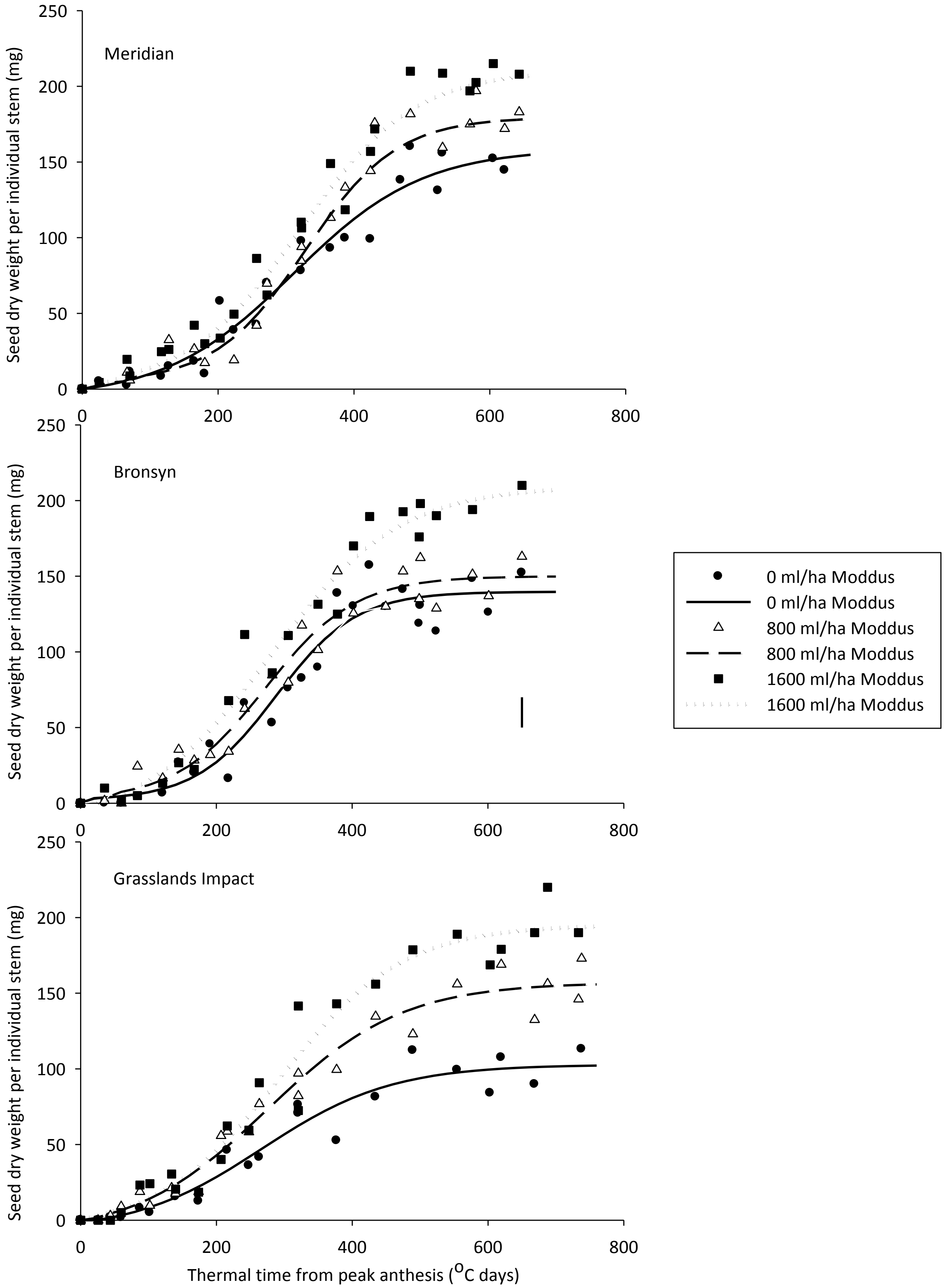



**Figure 5.3.** Seed growth expressed as a function of thermal time after peak anthesis for three rates of Moddus (a.i. 250 g/l Trinexapac ethyl) applied to three cultivars of perennial ryegrass, average of two sowing dates (SD1 =1 April 2008, SD2 =14 May 2008) at Lincoln University, Canterbury in the 2008/09 growing season. Error bar = S.E.M for Cultivar*Moddus at final harvest.

The influence of Moddus on maximum seed weight/head ('C') was large at 70 mg/head difference between 0 and 1600 ml/ha (Table 5.9).

Table 5.9. Maximum curve values ('C') for seed growth as influenced by the plant growth regulator, Moddus (250 g/l Trinexapac ethyl), for the cultivar by Moddus interaction in perennial ryegrass crops grown at Lincoln University, Canterbury in the 2008/09 growing season.

| Treatment | 'C' parameter values for Moddus PGR (ml/ha) | | |
|---|---|---|---|
| | 0 | 800 | 1600 |
| 'Meridian' | 155 | 199 | 223 |
| 'Bronsyn' | 153 | 155 | 208 |
| 'Grasslands Impact' | 102 | 158 | 194 |
| Mean | 137 | 171 | 208 |
| | Moddus | Cultivar*Moddus interaction | |
| P value | <0.001 | 0.522 | |
| S.E.M | 11.6 | 13.79 | |
| $LSD_{0.05}$ | 33.9 | within cultivar 58.8<br>between cultivar 57.5 | |

There was no sowing date, cultivar or Moddus effect (P<0.05) on the lag phase (time to 5% of final dry weight) at 150 ± 9.8 °C days or the duration of the linear phase (294 ± 14°C days).

The application of Moddus increased (P<0.001) the slope during the linear phase of seed growth. The 1600 ml/ha Moddus rate had a mean slope of 0.738 ± 0.041 mg/°C day which was different from the 800 ml/ha and 0 ml/ha treatments of 0.587 mg/°C day and 0.499 respectively ($LSD_{0.05}$ =0.121). There was a trend for cultivar (P<0.065) to influence the slope of the linear phase (Table 5.10).

Time to 95% of final seed weight and the duration of seed fill were unaffected (P<0.05) by sowing date, cultivar and Moddus or any interactions. There were trends that cultivar (P<0.069) and Moddus (P<0.052) affected time to 95% of final seed weight. The thermal time requirement for 95% seed fill was 443 ± 13.5 °C days.

**Table 5.10.** Seed growth rate per individual head (mg/°C day) for three cultivars of perennial ryegrass when treated with the three rates of Moddus (a.i. 250 g/l Trinexapac ethyl), grown at Lincoln University in the 2008/09 growing season.

| Treatment | Seed growth rate per individual head (mg/°C day) | | | |
|---|---|---|---|---|
| | Moddus rate (ml/ha) | | | |
| | 0 | 800 | 1600 | Cultivar Mean |
| 'Meridian' | 0.481 | 0.642 | 0.676 | 0.599 |
| 'Bronsyn' | 0.559 | 0.593 | 0.910 | 0.688 |
| 'Grasslands Impact' | 0.457 | 0.527 | 0.628 | 0.537 |
| Mean | 0.499 b | 0.587 b | 0.738 a | 0.608 |
| | Moddus | Cultivar | | |
| P value | <0.001 | 0.065 | | |
| S.E.M | 0.041 | 0.038 | | |
| $LSD_{0.05}$ | 0.121 | 0.124 | | |

## 5.6 Changes in stem dry weight following anthesis

### 5.6.1 Materials and methods

Stems were collected as described in Chapter 3. Briefly, from anthesis through to seed shedding, a 0.3 m length of 3 drill rows was cut every 3 – 4 days and 20 main stems selected from it. Stems were separated into stem and seed head by cutting at the basal node of the seed head. Stems had all leaves and any daughter tillers removed, they were then oven dried at 60 °C to a constant weight and weighed to four decimal places. The stem weights reported here are the dry weights of the stem from ground level to the base of the seed head with the dry weight of the rachis added.

#### Curve fitting

Stem weight was converted to mg per individual stem. The starting stem weight was set to zero by subtracting the weight of stem at anthesis from all subsequent measurements, therefore any negative values mean stem weight is less than it was at anthesis. A quadratic polynomial curve was fitted to the individual plot data using the Genstat 14 statistical package. If the quadratic curve did not have an $R^2$ value above 70% then a cubic polynomial was fitted. Fitted lines were then visually assessed for fit and physiological sense. In particular that they follow an expected forward progression. The curve which then fitted the data for least complexity was chosen. For all plots a quadratic curve was fitted (Equation 5.5).

Equation 5.5 $Y = ax^2 + bx + c$

Where 'a' and 'b' are curve parameters and 'X' is any value on the x-axis.

'c' is a constant.

Following curve fitting the parameters 'a' 'b' and 'c' were compared for differences by analysis of variance (ANOVA) with means separation achieved using the least significant difference (LSD) test. Maximum stem weight was estimated for all individual plots by substituting 'X' in Equation 5.5 with values of thermal time in 5°C day increments. Stem weight at maturity was established for individual plots by substituting X for the °C day corresponding to 99% of final seed weight. The values generated were then subjected to ANOVA and LSD analysis.

At final harvest 20 stems per plot were cut at ground level and the length of each internode measured. Internode 1 refers to the internode between the ear and flag leaf (final leaf) node, internode 4 was the internode closest to ground level.

## 5.7 Stem weight at anthesis

The stem dry weight at anthesis, defined as dry weight of stems cut at ground level to the base of the seed head plus rachis weight, was affected ($P<0.05$) by a sowing date by Moddus interaction. For crops sown 1 April, Moddus had no effect on stem weight (317 mg) (Table 5.11). When sowing was delayed to 14 May, there was a 62 mg decrease in stem weight between 0 ml/ha Moddus and 1600 ml/ha (299 mg) (Table 5.10).

**Table 5.11**. Individual stem weight at anthesis for perennial ryegrass crops sown on two dates and treated with three rates of Moddus (a.i. 250 g/l Trinexapac ethyl) at Lincoln University during the 2008/09 growing season.

| Sowing date | Individual stem weight (mg) | | | Mean |
|---|---|---|---|---|
| | Moddus (ml/ha) | | | |
| | 0 | 800 | 1600 | |
| 1 April | 308 | 324 | 320 | 317 |
| 14 May | 361 | 330 | 299 | 330 |
| Moddus mean | 334 | 327 | 309 | |
| Effect | P value | SEM | | $LSD_{0.05}$ |
| Sow date*Moddus | 0.049 | 14.1 | within sow date | 41 |
| | | | between sow date | 44 |

### 5.7.1 Stem weight accumulation following anthesis as a function of days after peak anthesis

Stem weight increased ($P<0.001$) following anthesis for all treatments. Maximum stem weight was calculated for each plot from the fitted quadratic equation (relative to stem weight at anthesis). The quadratic fit to data expressed in days after anthesis was average (mean $R^2$ = 72). Time to maximum stem weight was affected ($P<0.01$) by cultivar and Moddus (Table 5.12). For Moddus the 0 and 800 ml/ha rates were similar at 22.7 ± 1.16 and 24.1 days respectively compared with 28.6 days for the 1600 ml/ha rate ($LSD_{0.05}$ = 3.39).

**Table 5.12.** Time (days) to maximum stem weight (relative to stem weight at peak anthesis) as derived from quadratic polynomial regression for three cultivars of perennial ryegrass and three Moddus (a.i. 250 g/l Trinexapac ethyl) rates grown at Lincoln University in the 2008/09 growing season.

| Cultivar | Moddus Rate (ml/ha) | | | Cultivar mean |
|---|---|---|---|---|
| | 0 | 800 | 1600 | |
| 'Meridian' | 27.2 | 31.7 | 31.5 | 30.1 a |
| 'Bronsyn' | 22.2 | 19.0 | 29.2 | 23.4 b |
| 'Grasslands Impact' | 19.0 | 21.7 | 25.2 | 21.9 b |
| Moddus mean | 22.8 b | 24.1 b | 28.6 a | |
| Effect | P Value | SEM | $LSD_{0.05}$ | |
| Cultivar | 0.004 | 15.7 | 4.06 | |
| Moddus | 0.003 | 11.6 | 3.39 | |

When stem weight was analysed as a function of days after peak anthesis, maximum stem weight was influenced by a cultivar by Moddus ($P<0.05$) interaction. For 'Impact' and 'Meridian' the 800 ml/ha Moddus treatment gave the maximum stem weight compared with 'Bronsyn' where the 1600 ml/ha Moddus showed the maximum stem weight (Table 5.13). Final stem weight was affected by the cultivar by Moddus interaction ($P<0.01$).

**Table 5.13.** Maximum stem weight, days to maximum stem weight and final stem weight (relative to stem weight at peak anthesis) as derived from quadratic polynomial regression for three cultivars of perennial ryegrass and three Moddus (a.i. 250 g/l Trinexapac ethyl) rates grown at Lincoln University in the 2008/09 growing season.

| Cultivar | Moddus (ml/ha) | Maximum stem weight (mg) | Final stem weight * (mg) | difference (max – final) |
|---|---|---|---|---|
| 'Meridian' | 0 | 133 | 82 | 51 |
| | 800 | 217 | 189 | 28 |
| | 1600 | 167 | 147 | 20 |
| 'Bronsyn' | 0 | 144 | 39 | 105 |
| | 800 | 107 | -5 | 112 |
| | 1600 | 167 | 70 | 97 |
| 'Grasslands Impact' | 0 | 66 | -9 | 75 |
| | 800 | 115 | 31 | 84 |
| | 1600 | 100 | 65 | 35 |
| P value | | 0.036 | 0.0228 | |
| SEM | | 18.44 | 23.7 | |
| $LSD_{0.05}$ | within cultivar | 54 | 69 | |
| | different cultivar | 69 | 76 | |

* Note; positive values are heavier than stems from the same treatments at anthesis while negative values are lighter

## 5.7.2 Stem weight accumulation following anthesis as a function of thermal time

Stem dry weight increased (P<0.001) following anthesis to a maximum followed by a decrease to the final weight at harvest (Figure 5.4). The weight of stems at harvest relative to anthesis (net change) was affected by Moddus (P<0.01) and cultivar (P>0.05) but none of their interactions. On average all stems gained weight,those treated with Moddus were 74 mg heavier at final harvest than at anthesis compared with 18 mg where 0 ml/ha of Moddus was applied. Stems of 'Meridian' gained 106 mg following anthesis compared with 30 mg for both 'Grasslands Impact' and 'Bronsyn'. Data for the cultivar by Moddus interaction shown in Table 5.15 for clarity and reference to Figure 5.4.

**Table 5.14** Final stem weight relative to stem weight at anthesis (net change) for three cultivars of perennial ryegrass and three Moddus (a.i. 250 g/l Trinexapac ethyl) rates grown at Lincoln University, Canterbury in the 2008/09 growing season.

| Cultivar | Moddus Rate (ml/ha) | | | Cultivar mean |
|---|---|---|---|---|
| | 0 | 800 | 1600 | |
| 'Meridian' | 52 | 163 | 104 | 106 a |
| 'Bronsyn' | 7.9 | 6 | 60 | 37 b |
| 'Grasslands Impact' | -5.24 | 65 | 51 | 25 b |
| Moddus mean | 18 b | 78 a | 72 a | |
| Effect | P value | SEM | $LSD_{0.05}$ | |
| Cultivar | 0.025 | 18.0 | 59 | |
| Moddus | 0.004 | 12.4 | 36 | |
| Cultivar*Moddus | 0.081 | 21.4 | NS | |

Maximum stem weight following anthesis was affected by a cultivar by Moddus (P<0.01) interaction (Table 5.15). For 'Impact' and 'Meridian' the 800 ml/ha Moddus treatment gave the maximum stem weight compared with 'Bronsyn' where 1600 ml/ha Moddus showed maximum stem weight (Table 5.15).

There was no difference in the stem dry weight lost following the maximum stem weight after anthesis, largely due to variability between plots where the coefficient of variation (CV%) was 33%, the grand mean was 84 mg (Table 5.15). The thermal time to maximum stem weight was affected by cultivar (P<0.001). The stems of 'Bronsyn' reached maximum weight at 309 ± 27 °C days which was quicker than 'Grasslands Impact' and 'Meridian', which were similar at 379 and 430 °C days respectively ($LSD_{0.05}$ = 63).

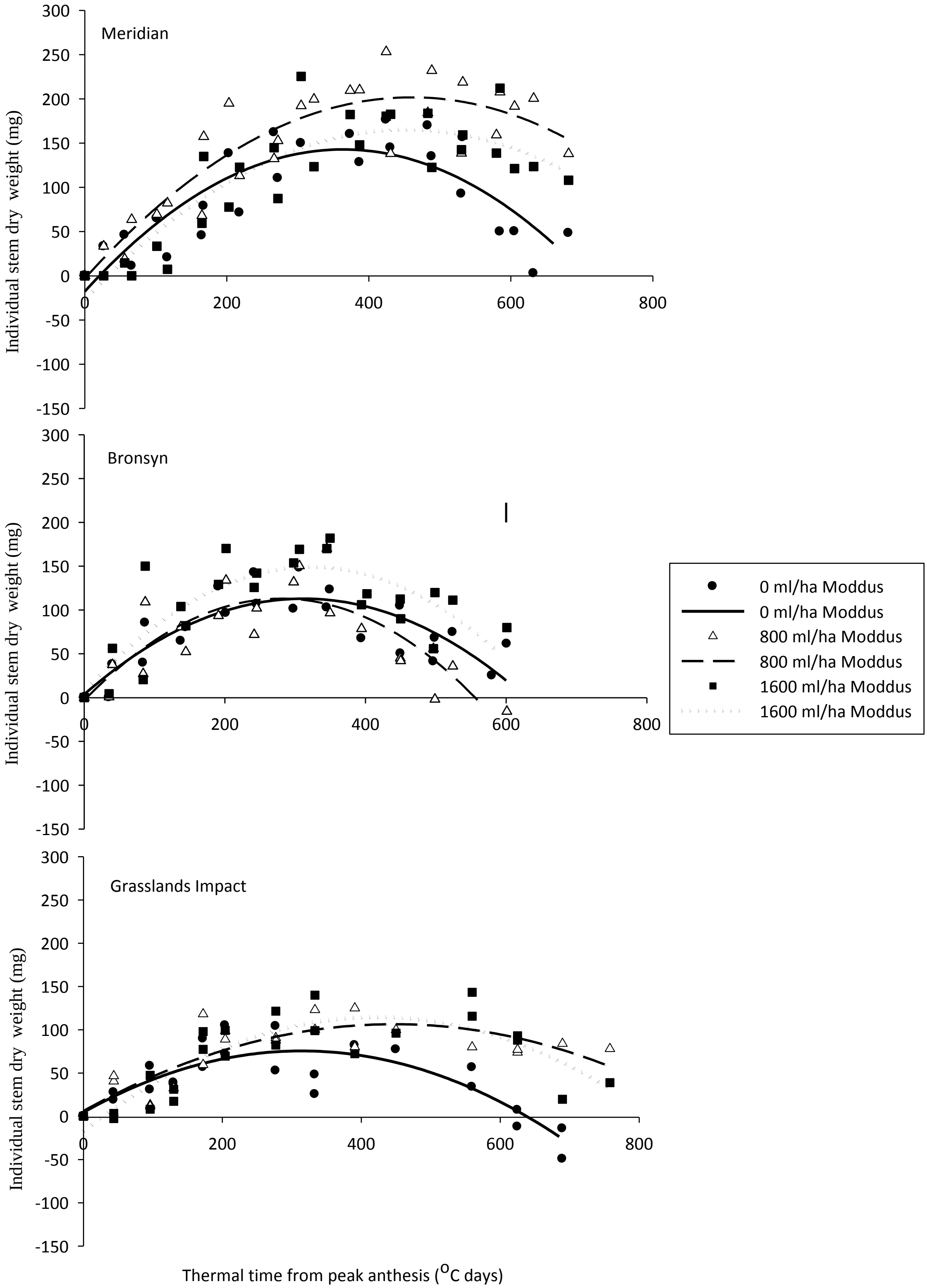



**Figure 5.4.** Changes in stem dry weight post peak anthesis for three perennial ryegrass cultivars with three Moddus (a.i. 250 g/l Trinexapac ethyl) rates, Lincoln University, Canterbury 2008/09. Error bar = S.E.M for Cultivar*Moddus for the final harvest weights.

**Table 5.15.** Maximum stem weight increase, the change in stem weight from anthesis to harvest and the difference between maximum weight gain and harvest weight as derived from cubic polynomial regression for three cultivars of perennial ryegrass and three Moddus (a.i. 250 g/l Trinexapac ethyl) rates, Lincoln University, Canterbury, 2008/09 growing season.

| Cultivar | Moddus (ml/ha) | Maximum stem weight increase (mg) | Stem weight at harvest relative to anthesis (mg) * | difference (max – final) (mg) |
|---|---|---|---|---|
| 'Meridian' | 0 | 139 | 52 | 87.0 |
| | 800 | 224 | 163 | 60.6 |
| | 1600 | 168 | 104 | 64.0 |
| 'Bronsyn' | 0 | 128 | 8 | 120 |
| | 800 | 135 | 6 | 129 |
| | 1600 | 160 | 60 | 100 |
| 'Grasslands Impact' | 0 | 70 | -5 | 75.0 |
| | 800 | 102 | 65 | 37.0 |
| | 1600 | 132 | 51 | 81.0 |
| | | cultivar*Moddus | cultivar*Moddus | difference |
| P value | | 0.046 | 0.041 | 0.259 |
| SEM | | 16.2 | 21.4 | 28.4 |
| $LSD_{0.05}$ | within cultivar | 47.3 | 62.5 | |
| | between cultivar | 63.3 | 77.6 | NS |

* Note; positive values are heavier than stems from the same treatments at anthesis while negative values are lighter

The percentage weight change of stems from anthesis to harvest (final weight/anthesis weight) was affected by Moddus (P<0.01) and cultivar (P<0.05), there was a trend (P<0.065) for an interaction between Moddus and cultivar. Where Moddus was applied, stems at harvest were 25% heavier than at anthesis compared with 5% where no Moddus was applied (Table 5.16). Stems of 'Meridian' were 34% heavier than at anthesis compared with 'Bronsyn' and 'Grasslands Impact' which were similar at 10% heavier than anthesis.

**Table 5.16.** Percentage change in weight of stems (stem cut at ground level and includes rachis but not seed/spikelets) for three cultivars of perennial ryegrass and three Moddus (a.i. 250 g/l Trinexapac ethyl) rates grown at Lincoln University in the 2008/09 growing season.

| Cultivar | Moddus Rate (ml/ha) | | | Cultivar mean |
|---|---|---|---|---|
| | 0 | 800 | 1600 | |
| 'Meridian' | 15 | 54 | 33 | 34a |
| 'Bronsyn' | 3.3 | 1.5 | 20 | 8 b |
| 'Grasslands Impact' | -2.9 | 21 | 18 | 12b |
| Moddus mean | 5b | 25a | 24a | |
| Effect | P value | SEM | $LSD_{0.05}$ | |
| Cultivar | 0.023 | 5.6 | 18.3 | |
| Moddus | 0.003 | 4.2 | 12.2 | |
| Cultivar*Moddus | 0.06 | 7.2 | NS | |

## 5.8 Stem length

Moddus was the only treatment to influence (P<0.05) total stem or internode length (Table 5.17). Total stem length was reduced (P<0.001) by Moddus application from 0.75 ± 0.012 m to 0.66 m at 800 and 0.60 m at 1600 ml/ha Moddus ($LSD_{0.05}$ = 3.64). On average, 0.098 m of height was removed per 1000 ml/ha of Moddus applied.

**Table 5.17.** Total stem, seed head and internode lengths (1 = below the head, 4 = closest to the ground) for three rates of Moddus, average of three perennial ryegrass cultivars ('Meridian', 'Bronsyn' and 'Grasslands Impact') and two sowing dates, 2008/09 growing season, Lincoln University, Canterbury.

| Moddus parameter | Total stem length (m) | Internode length (m) | | | | |
|---|---|---|---|---|---|---|
| | | Seed head | 1 | 2 | 3 | 4 |
| 0 ml/ha | 0.75 a | 0.204 a | 0.233 a | 0.135 a | 0.105 a | 0.073 a |
| 800 ml/ha | 0.66 b | 0.185 b | 0.197 b | 0.124 ab | 0.095 a | 0.050 b |
| 1600 ml/ha | 0.60 c | 0.167 c | 0.187 b | 0.113 b | 0.075 b | 0.058 b |
| P value (Moddus) | <0.001 | <0.001 | <0.001 | 0.015 | <0.001 | 0.02 |
| S.E.M | 0.012 | 0.005 | 0.008 | 0.005 | 0.005 | 0.004 |
| Grand mean | 0.668 | 0.185 | 0.206 | 0.124 | 0.093 | 0.063 |
| $LSD_{0.05}$ | 0.036 | 0.015 | 0.024 | 0.014 | 0.014 | 0.011 |

### 5.8.1 Stems with vegetative daughter tillers at harvest.

Moddus was the only treatment to influence (P<0.05) the percentage of stems which had vegetative daughter tillers (Table 5.18). There was a trend (P<0.07) for a cultivar influence but none of the interactions. The 1600 ml/ha Moddus rate had fewer main stems with vegetative daughter tillers (3.5%) at final harvest compared with Moddus applied at 800 ml/ha (14.8%) and the untreated control (34.4%). The Moddus by cultivar interaction is presented in Table 5.18.

**Table 5.18.** Percentage of mainstems at final harvest with vegetative daughter tillers growing from the above ground nodes for three cultivars of perennial ryegrass treated with three rates of Moddus (a.i. 250 g/l Trinexapac ethyl) grown at Lincoln University, Canterbury in the 2008/09 growing season.

| Cultivar | Percentage stems with daughter tillers (%) | | | Cultivar mean |
|---|---|---|---|---|
| | Moddus rate (ml/ha) | | | |
| | 0 | 800 | 1600 | |
| 'Meridian' | 31.7 | 9.67 | 2.33 | 14.56 |
| 'Bronsyn' | 36.5 | 23.8 | 3.00 | 21.1 |
| 'Grasslands Impact' | 35.2 | 11.0 | 5.17 | 17.1 |
| Mean | **34.4 a** | **14.8 b** | **3.5 c** | |
| Effect | Pvalue | SEM | $LSD_{0.05}$ | |
| Moddus | <0.001 | 2.58 | 7.53 | |
| Cultivar | 0.069 | 1.69 | NS | |

Note. Bold figures indicate Moddus affect.

## 5.9 Seed head and stem interactions.

All treatments (sowing date, cultivar and Moddus) showed (P<0.05) maximum stem weight occurred approx. 77 ± 24 °C days later (P<0.05) than 50% seed filling (parameter 'M' in seed growth curves).

For 'Meridian', the 800 ml/ha Moddus rate produced the heaviest stems at both maximum weight and final harvest, however the 1600 ml/ha Moddus rate produced the highest seed yield and the shortest stems. For 'Bronsyn' the 0 and 800 ml/ha Moddus treatments produced similar seed and stem weights following anthesis compared with the 1600 ml/ha Moddus rate which produced heavier stems and the highest seed yield. For 'Grasslands Impact' the 1600 ml/ha Moddus treatment produced the heaviest stems 400 °C days after anthesis but at harvest the 800 ml/ha Moddus treatment had heavier stems. However the 1600 ml/ha Moddus rate produced the highest seed yield. For 'Bronsyn' the heaviest stems at harvest produced the highest seed yield compared with 'Meridian' and 'Grasslands Impact' where the lighter stems from the 1600 ml/ha Moddus treatment (54 mg lighter than 800 ml/ha in Bronsyn) produced the greatest seed weight.

In all cultivars the shortest stems produced the highest seed yield.

## 5.10 Harvest index

### Analysis methods

Harvest index was calculated for individual seed head and stem data by Equation 5.6 for data collected at three or four day intervals.

Equation 5.6 HI = seed weight/(seed weight + stem weight + seed head at anthesis)

Thus, harvest index is defined only as the increase in seed components following anthesis e.g. embryo and endosperm, as the lemma and palea which form part of the ryegrass seed are included in the seed head weight at anthesis. For each stem all leaf and daughter tillers were removed. Linear regression (Equation 4.1) coefficients were generated for individual plots using Genstat 14. Coefficients were analysed using analysis of variance for treatment differences. The location where the regression line crossed the x-axis was found by substituting zero for 'y' in Equation 4.2, thus gaining an estimate of the lag phase for seed growth.

### 5.10.2 Mainstem harvest index

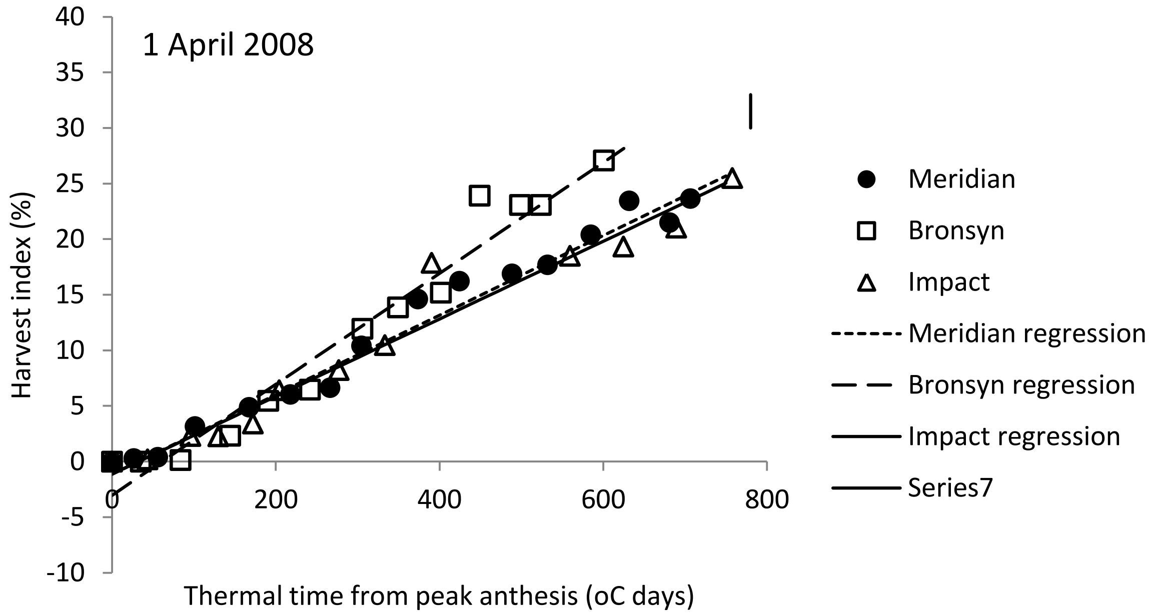
The final harvest index on the main stems was affected ($P<0.01$) by sowing date and Moddus but not cultivar or any interactions. The final harvest index for 14 May (35 ± 1.06%) was greater than sowing on 1 April (25%) ($LSD_{0.05}$= 3.66). For Moddus, 800 ml/ha (30 ± 1.55%) was similar to 0 ml/ha (26%) and 1600 ml/ha (34%) which were different from each other ($LSD_{0.05}$ = 5.48).

### 5.10.3 Change in harvest index per °C day

The change in harvest index per °C day (*d*HI/*d*t) was linear (Figure 5.5) and affected by a sowing date by cultivar interaction ($P<0.05$) and the main effect of Moddus ($P<0.05$). Delaying sowing from 1 April to 14 May increased ($P<0.05$) *d* HI/*d*t in 'Meridian' and 'Grasslands Impact' but not 'Bronsyn' (Table 5.19).

**Table 5.19.** Change in harvest index per day for three perennial ryegrass cultivars sown on two dates at Lincoln University, Canterbury in the 2008/09 growing season.

| Cultivar | Sowing date | | Change | |
|---|---|---|---|---|
| | 1 April | 14 May | | |
| 'Meridian' | 0.036 | 0.063 | 0.027 | |
| 'Bronsyn' | 0.050 | 0.059 | 0.009 | |
| 'Grasslands Impact' | 0.035 | 0.050 | 0.010 | |
| | P value | SEM | | $LSD_{0.05}$ |
| Sowing date*Cultivar | 0.019 | 0.003 | Within sowing date | 0.0088 |
| | | | Between dates | 0.0097 |



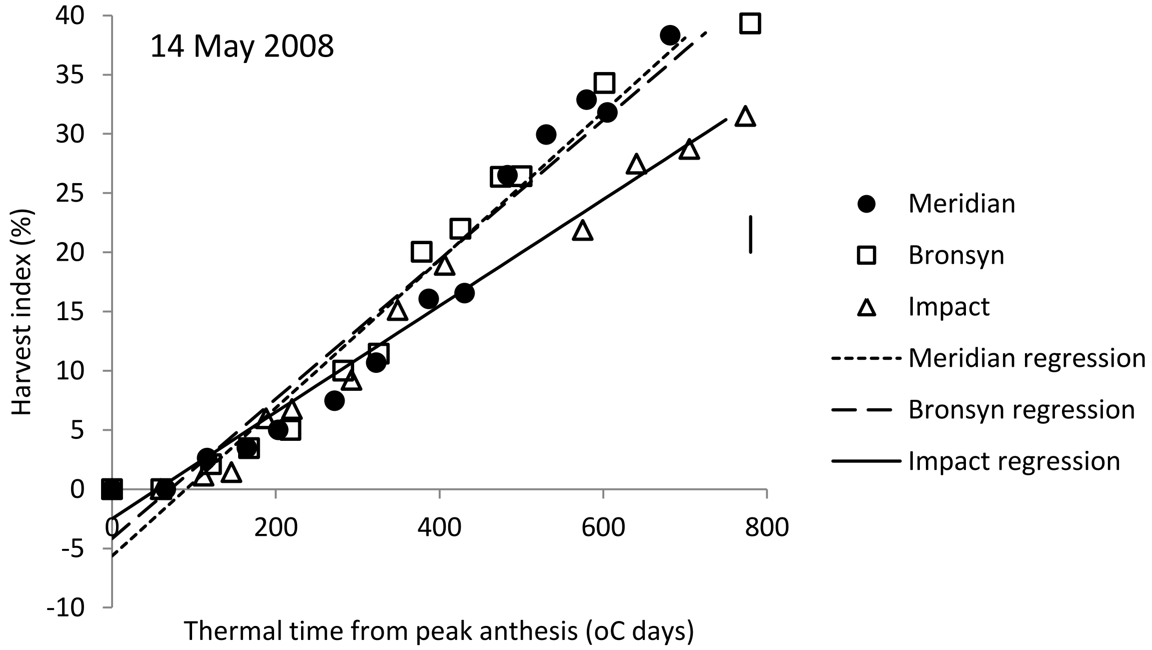




**Figure 5.5.** Change in harvest index per °C day for three cultivars of perennial ryegrass following peak anthesis from two sowing dates at Lincoln University, Canterbury in the 2008/09 growing season. Error bar = Standard error of the mean for sowing date*cultivar interaction at the final harvest index measurement.

For Moddus, dHI/dt was higher (P<0.05) when 1600 ml/ha (0.053 ± 0.002 mg/°C day) was applied compared with 800 ml/ha (0.047) which was similar to 0 ml/ha (0.043 mg/°C day) (Table 5.20). There was no treatment effect (P<0.05) on the x-axis intercept, suggesting the lag phase of seed growth was the same for all cultivars (56°C days). Moddus was the only main effect treatment to influence (P<0.05) the y-axis intercept, there were no interactions. The y-axis intercept for 800 ml/ha Moddus (3.04 ± 0.435%) was similar to both 0 ml/ha (-2.05%) and 1600ml/ha (-3.73%) which were different from each other ($LSD_{0.05}$ = 1.27).

**Table 5.20.** Change in harvest index/°C day for three Moddus (a.i. 250 g/l Trinexapac ethyl) rates, average of three cultivars and two sowing dates at Lincoln University, Canterbury in the 2008/09 growing season.

| Moddus rate (ml/ha) | Change in harvest index/°C day |
|---|---|
| 0 | 0.043 |
| 800 | 0.047 |
| 1600 | 0.053 |
| P value | 0.005 |
| SEM | 0.002 |
| LSD | 0.005 |

## 5.10.4 Change in harvest index per calender day

The change in harvest index per day was linear (Figure 5.6) and affected (P<0.05) by sowing date and Moddus rate (Table 5.21). There was also a trend (P= 0.0617) for a cultivar effect on the slope. There was no treatment effect (P<0.05) on the constant (y-axis intercept) or the x-axis intercept, suggesting the lag phase of seed growth was the same for all cultivars. The change in harvest index per day for 1 April sowing was slower (P<0.05) than the 14 May sowing date. The harvest index at the final harvest followed the same trend.

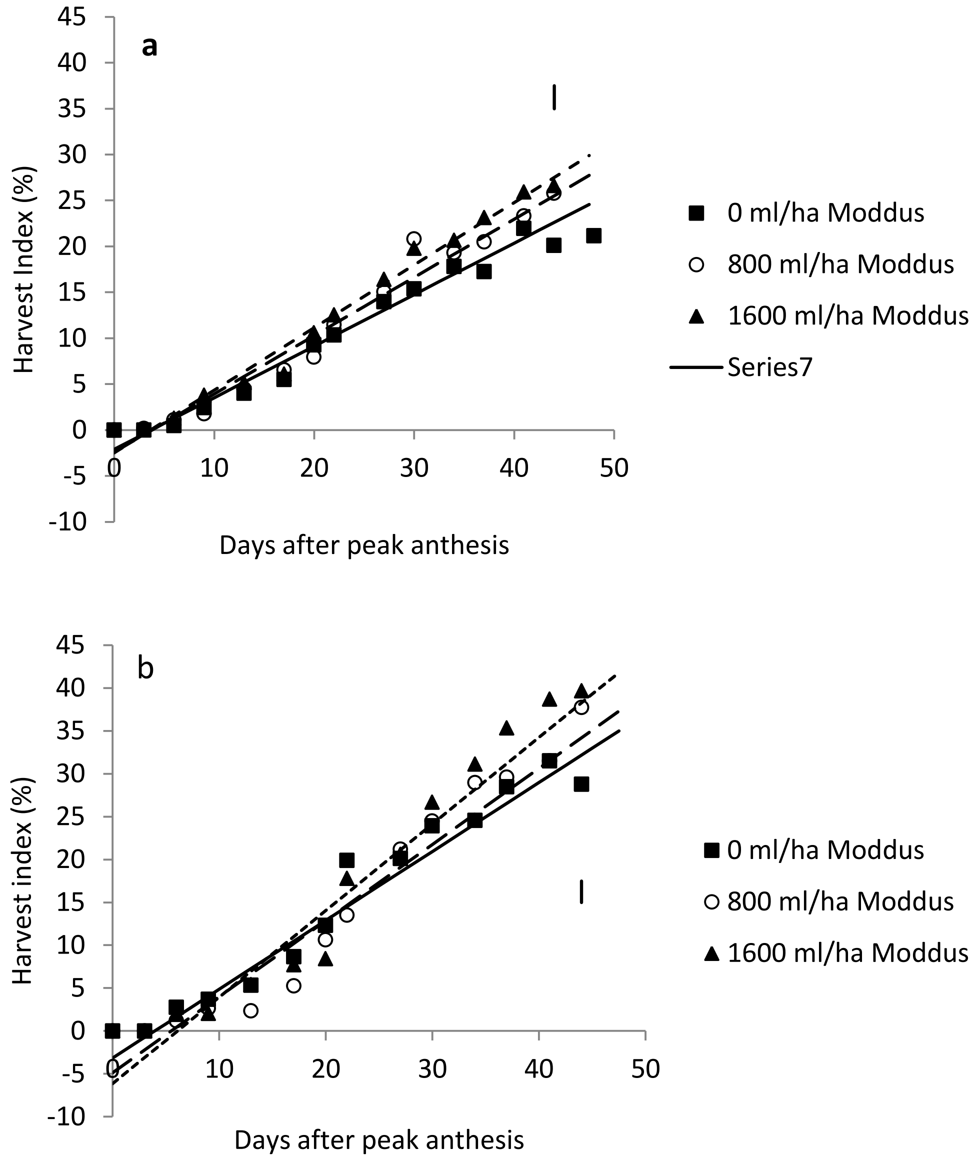



**Figure 5.6.** Change in harvest index per day for perennial ryegrass (*Lolium perenne*) following peak anthesis for two sowing dates (a; 1 April 2008 and b; 14 May 2008) and three Moddus rates (a.i. 250 ml/l Trinexapac ethyl) ( ——0 ml/ha, — — 800 ml/ha and --- 1600ml/ha Moddus), average if three cultivars; 'Meridian', 'Bronsyn' and 'Grasslands Impact'. Error bar = standard error of the mean for sowing date*Moddus interaction at the final harvest index measurement

**Table 5.21.** Rate of change in harvest index/day (slope of linear regression) of main stems when assessed at 3-4 day intervals for two sowing dates and three Moddus (a.i. 250 g/l Trinexapac ethyl) rates, average of three perennial ryegrass cultivars, Lincoln University, Canterbury 2008/09 growing season.

| Change of harvest index/day (%) | | | | |
|---|---|---|---|---|
| Sowing date | Moddus rate (ml/ha) | | | Sowing date mean |
| | 0 | 800 | 1600 | |
| 1st April | 0.5614 | 0.6346 | 0.6819 | 0.6259 |
| 14th May | 0.8030 | 0.8878 | 1.0090 | 0.8999 |
| Moddus mean | 0.6822 | 0.7612 | 0.8454 | 0.7629 |

| | P value | SEM |
|---|---|---|
| Sow date | 0.0124 | 0.0218 |
| Cultivar | 0.0617 | 0.034 |
| Moddus | 0.0157 | 0.036 |
| Sow date*Moddus | | |

## 5.11 Discussion

The timing of main stem anthesis was influenced by a cultivar by sowing date interaction (Figure 5.1; Table 5.1). The approximate 30 day difference between cultivars was expected based on their cultivar classification (Agriseeds, 2006). This gave rise to different environmental conditions for seed growth. The timing of reproductive initiation in perennial ryegrass is determined by vernalisation and day-length (Cooper, 1949). It is generally accepted that later flowering cultivars require a longer day-length (shorter night) relative to earlier flowering cultivars to induce stem extension. The application of Moddus delayed ($P<0.05$) anthesis by one day, however while of interest in the current experiment, on farm this will also be of little practical importance (Table 5.2). Moddus reduced ($P<0.05$) stem length (Table 5.17) through reducing internode length and therefore will possibly affect the capacity of the stem to store assimilate i.e. shorter stems may have less storage ability compared with longer stems (Bonnett and Incoll, 1992). In a sink limited crop less stem storage is beneficial as the stem stops competition for assimilate earlier allowing for transport to developing seeds (Austin *et al*., 1980)

Following anthesis seed growth followed a sigmoidal growth pattern for the three perennial ryegrass cultivars ('Meridian', 'Bronsyn' and 'Grasslands Impact') when described over both days and thermal time (base temperature 0 °C). Seed growth curves for both days and thermal time were symmetrical about the inflection point and therefore suited to the Logistic curve (Figure 5.2, Figure 5.3). This is in contrast to Warringa *et al*. (1998) who used an asymmetrical growth curve (Gompertz function) to describe seed growth in the Dutch cultivar 'Barlet'. However, Warringa *et al*. (1998) did not measure seed weight until approximately 14 days after anthesis, and therefore 'assumed' the shape of the growth curve prior to mid seed fill.

All parameter estimates for the Logistic functions (inflection point, curve shape and curve maximum) were affected by cultivar when expressed over days after anthesis (Table 5.3) while Moddus affected the fitted maximum seed weight only (Table 5.4, Table 5.9). When compared with thermal time the cultivar influence on the inflection point disappeared (Table 5.8) while the other effects remained the same. Specifically, cultivar still influenced the shape of the seed growth curve and both cultivar and Moddus influenced the maximum seed weight (Table 5.8). The consistent inflection point relative to thermal time, suggests thermal time was a more accurate descriptor of seed growth. This was supported by the time to 95% seed weight where 'Meridian' took eight days longer ($P<0.05$) than 'Bronsyn' and 'Grasslands Impact' (where expressed in days after anthesis) (Table 5.7) but was similar in thermal time ($P=0.12$). The use of thermal time should allow for valid comparisons between locations and seasons when there are differences in temperature regimes. Overall these results are the first to show that seed development in perennial ryegrass was consistent with other monocotyledonous species. Based on results for wheat, Monteith (1977) concluded that kernel filling should be related to thermal time rather than days. Not surprisingly, it appears seed growth for perennial ryegrass should also be expressed against thermal time.

The seed growth pattern had three distinct phases (Figure 5.3), lag, linear and maturation. This was consistent with the work by Warringa *et al*., (1998) on perennial ryegrass and similar to previous studies in cereals ( Section 2.4.1). The mean lag phase was 11 days and the mean duration of the linear phase of seed growth was 16 days. Both were unaffected ($P<0.05$) by any of the imposed treatments. Meridian (33 days) took longer ($P<0.05$) to reach 95% of final seed weight compared with 'Bronsyn' and 'Grasslands Impact' (25 days) (Table 5.7). The duration of growth in days is supported by Warringa *et al*. (1998) who found the duration of growth to be 28 days for the Dutch cultivar 'Barlet'.

In thermal time the duration of the lag phase was the similar for all cultivars at 150 °C days. This is longer than the 100 °C days reported for wheat cultivars (Loss *et al*., 1989). However, Bauer *et al*. (1985) demonstrated variability in wheat where more modern cultivars had a shorter lag phase, approximately 140 °C days, compared with lower yielding, older cultivars, where the lag phase was 210 °C days. Crampton (1998) demonstrated the lag phase in oats was longer than wheat at 232 °C days. In these cases, the total duration of seed filling remained constant for both cultivars and treatments, about 750 °C days in wheat (Loss *et al*., 1989; Bauer *et al*., 1985) and 690 °C days in oats (Crampton 1998). Thus, a shorter lag phase gives an increase in the duration of the linear phase of seed growth. Physiologically the lag phase is characterised by rapid cell division until the potential cell number is reached and therefore the potential grain size (Brocklehurst, 1977). The lag phase finishes with the influx of protein and starch (see section 2.7.1). Differences in the length of the lag phase therefore determine the potential seed size (number of cells) within a crop and the onset of dry weight deposition (the linear phase of seed growth). In wheat, most modern cultivars have a dwarfing gene, e.g. *Rht2* which leads to a greater number of grains produced/$m^2$ (Austin *et al*. 1989). The higher grain population combined with the shorter lag phase of more modern wheat cultivars has been reported to reduce final grain size (Borrel *et al*. 1991; Miralles and Slafer 1996). The reduction in grain size is likely to be a combination of a lower cell number (reduced lag phase) and perhaps increased competition of assimilate between an increased number of individual grains.

Therefore selection for a shorter lag phase has the potential to improve seed yield in perennial ryegrass through extension of the linear phase of seed filling. A reduction in potential seed size (shorter lag phase) may also improve the conversion of under size seed to first grade seed if it means more assimilate can be distributed to the smaller seeds (if they themselves have the capacity to become heavier).

The duration of the linear phase of seed growth, defined as the time from 5 to 95% of final seed weight was similar for all sowing date, cultivar and Moddus treatments at 294 °C days. This duration was short compared with that of wheat at 550 °C days (Loss *et al*., 1989). During the linear phase of growth 90% of final seed dry weight was accumulated with no difference in the overall duration. Therefore, the 0.239 mg/°C day difference in seed filling rate/head was the main cause of seed yield differences between Moddus rates (Table 5.10). There was a trend (P<0.06) for Moddus to increase the rate of seed fill when expressed in days after anthesis (Table 5.6). This result suggests Moddus (or any other straw shortener) should be used to increase the rate of seed filling in perennial ryegrass. Moddus shortened all internodes, including the total length of the seed head.

The overall duration of seed filling appears constant in thermal time at 443 °C days. There was no difference between any of the treatments imposed. Recalculating the thermal time conditions from the greenhouse experiment of Warringa *et al*., (1998) shows a requirement of 532 °C days from anthesis to maturity for individual seeds. The differences reported between the two studies are likely to evolve from the use of individual seeds from anthesis compared with whole seed head and the definition of peak anthesis. Other variation could exist around the definition of maturity, possible genetic differences and the method and location of temperature recording. In the study by Warringa *et al*. (1998) plants were grown in a glasshouse where temperature was controlled but may have 'drifted' outside the targeted temperature depending on cooling capacity compared with field conditions in the current study.

For cultivar (P=0.065), differences in final seed weight per head can be attributed to changes in the slope of the linear phase of seed filling (Table 5.10;Figure 5.3). This result is similar to that shown by Crampton (1998) where the variation in individual seed size for primary and secondary kernel weight in oats was due to changes in the rate of filling, not the duration of the linear phase. Both the conversion of total seed yield to first grade seed yield and the thousand seed weight were greater for 'Meridian' and 'Bronsyn' compared with 'Grasslands Impact' (Table 4.9). Since the duration of seed filling was similar, this lends weight to the argument that the rate of filling between cultivars differed. Similar results have been shown for barley (Lee, 1991) and wheat (Sofield *et al*., 1977) where variation in individual seed weight within heads was due to the rate of linear growth. The final individual kernel weights of Lee (1991), Sofield *et al*. (1977) and Crampton (1998) support the idea that a kernels ability to compete for assimilate is due to its size relative to other kernels at any one time (Marcelis, 1996) i.e. kernels which were larger at anthesis were larger at maturity. Scott *et al*. (1983) showed that final kernel weight in barley was related to ovule size at anthesis, which is related to the order of differentiation at the apical meristem (Kirby, 1977; refer section 2.4). Therefore potential to store carbohydrate is a combination of ovary size at anthesis and the number of endosperm cells formed during the lag phase, while actual seed weight depends on filling the cells to capacity. Anslow (1964) showed seed weight depends on the position of the seed within the seed head. Seed weight decreased from the basal to distal spikelets and from basal to distal seeds within spikelets. Therefore seeds located further from the source of assimilate (stem) appeared to be lighter. Therefore the selection of a shorter rachis, as simulated by the application of Moddus, may increase the ability for the distal spikelets and seed to attract assimilate.

In barley, Lee (1991) indicated that, terminal kernels were disadvantaged by having a shorter growth duration compared with basal kernels. In perennial ryegrass, Warringa *et al*. (1998) demonstrated differences in seed filling duration within an individual spikelet where individual seeds matured at the same time, but anthesis varied by five days from the basal to the terminal spikelet. Within the individual spikelet of perennial ryegrass, the basal and central seeds grow at the same rate while the distal seeds grow at a slower rate (Warringa *et al*., 1998), possibly as a result of being located further from the assimilate source (Lee, 1991, Anslow, 1964). On a whole head basis the duration of seed filling was the same, therefore the rate of filling per head influenced the final yield per head (mg of DM/head). How the assimilate is partitioned within each individual head i.e. between individual spikelets followed by individual seeds, is likely to determine the final individual seed weight. With a trend for 'Meridian' and 'Bronsyn' to have a higher rate of seed filling per head, it appears that genetic improvement could be made to alter the rate of seed filling in perennial ryegrass, at least on a whole head basis. Where the stem was shortened by Moddus, the rate of seed filling was greater, therefore selection for shorter stem length may also improve the rate of seed filling.

The application of Moddus increased ($P<0.05$) the rate of seed fill by 0.239 mg/°C day for 1600 ml/ha compared with the 0 ml/ha control when described using thermal time. In all cultivars it was the shortest stems (ground level to tip of terminal spikelet) (Table 5.17) which produced the highest seed yield. Similar results have been described by Austin *et al*., (1980) for wheat where shorter stems of more modern cultivars increased grain yield through a higher number of seeds/$m^2$. The stem can be considered a storage organ for assimilate (Hay and Walker 1989). The stem imports assimilate from photosynthetic tissue where it can be stored, waiting for re-transportation to another location e.g. seed growth, respiration or release of new tillers, therefore the stem has the ability to be a net importer or exporter of assimilate post anthesis (Section 2.8). Moddus was the only treatment to reduce stem length, therefore possibly reduce the stem storage area for assimilate (Bonnett and Incoll, 1992).

For the cultivars 'Bronsyn' and 'Grasslands Impact', 1600 ml/ha of Moddus produced the highest seed yield and the greatest increase in stem dry weight (within the respective cultivar), relative to stem dry weight at anthesis (Table 5.11) i.e. these stems grew more weight than stems from other treatments in the period from anthesis to harvest (stem weight is defined as the dry weight of stems cut at ground level and again at the basal node on the seed head plus the weight of rachis, but not seed weight). In 'Meridian', the stems of the 800 ml/ha Moddus treatments gained the most weight from anthesis to harvest (Table 5.12, Table 5.13), however the 1600 ml/ha rate of Moddus produced the highest seed yield. The 'Meridian' response to Moddus suggests a sink limitation in, at least, the 800 ml/ha Moddus rate, due to the increase in stem weight (carbohydrate) which was not required for seed filling, even though the seed yields achieved were high. This suggests that the seed requirements for assimilate were less than the amount of assimilate imported into the stem from photosynthesis during the same time period. A similar trend was shown in the majority of plots where Moddus was applied. Trethewey and Rolston (2009) showed that in perennial ryegrass the increase in stem dry weight following anthesis was due to the deposition of both short and long chain carbohydrates in the stems, a result similar to Warringa and Marinissen (1997). The idea of a sink limitation is supported by the 1600 ml/ha Moddus treatment which produced stems that gained weight post anthesis and had the highest seed yields. Therefore the stems had transported some assimilate to developing seed (unless the head itself had produced 100% of its requirements), but are likely to have still contained assimilate that could be remobilised to seed. Therefore the stem was a net importer of assimilate during the seed filling period.

Moddus increased individual stem weight by 25% at harvest compared with anthesis (Table 5.14, Table 5.16). This is similar to that reported by Trethewey and Rolston (2009) who showed a 31% increase in stem dry weight during seed fill where 1250 ml/ha of Moddus had been applied. The gain in stem weight (maximum) following anthesis was affected by a cultivar by Moddus interaction. In 'Meridian' and 'Grasslands Impact' the stem weight increased as Moddus rate increased from 0 to 1600 ml/ha e.g. in 'Grasslands Impact', stems dry weight increased by 70 mg at 0 ml/ha Moddus compared with a 132 mg increase at 1600 ml/ha Moddus (Table 5.15). The timing of the maximum stem dry weight accumulation, following anthesis, was influenced by cultivar. In 'Bronsyn' this occurred earlier (310 °C days after anthesis) compared with 400 °C days after anthesis for both 'Meridian' and 'Grasslands Impact'. Given the time to 95% final seed weight was constant at 443 °C days, the stem competed for assimilate longer in 'Meridian' and 'Grasslands Impact' compared with 'Bronsyn'. The difference in dry weight from the post anthesis maximum until harvest showed a net loss of 84 mg in all treatments (Table 5.15). Concurrently the gain in seed weight following the period of maximum stem weight was approximately 20 mg on the 0 Moddus treatments and 45 mg in the 1600 ml/ha Moddus treatments. Therefore between 40 and 60 mg of weight was lost to other sinks which could potentially be used for seed growth e.g. respiration and release of new tillers. The overlap where the stem was competing directly with the head was approximately three quarters of the timeframe from anthesis until 95% of final seed dry weight. The stems in this experiment competed with the seed head for longer (approx. ¾ of seed filling) than those shown by Warringa and Marinissen (1997) where stems competed directly for assimilate up until mid seed fill.

For seed yield to be increased growers and plant breeders need to use all possible mechanisms to reduce the competiveness of the stem relative to the developing seed. This may be controlled genetically given the difference shown for 'Bronsyn' where the stem stopped competing 90°C days earlier than 'Meridian' and 'Grasslands Impact'. Other techniques may include selection for a stem with less storage capacity.

The release of daughter tillers is likely to be related to the timing and severity of lodging (Table 4.3). Plot lodging data (Figure 4.2 and Table 4.3) and the percentage of stems with late season daughter tillers (Table 5.18) suggest there were a higher percentage of tillers where regrowth had commenced in plots which lodged earlier and had a greater absolute lodging percentage at harvest. Similar results have been presented by Clemence and Hebblethwaite (1984) and Rolston *et al*. (2007b). The release of new tiller is dependent on a high or increasing red:far red light ratio at the base of the crop (Barnes and Bugbee, 1991) which would occur after lodging (for further in formation refer to section 2.3.7).

In carbohydrate studies for Italian and perennial ryegrass, Griffith (1992 and 2000) has shown a trend with an accumulation of carbohydrate in the lower internodes following anthesis. In the Trethewey and Rolston (2009) study the stem was dissected into individual internodes where all internodes continued to gain weight following head emergence until harvest, the only exception to this being the basal most node. Trethewey and Rolston (2009) commented that very little 'regrowth' was seen during the study. However, loss from the lower stem could be expected in a perennial species as the lower stem is likely to contain carbohydrate for the maintenance and growth of the root system.

Clemence and Hebblethwaite (1984) demonstrated that up to 24% of $^{14}C$ was allocated to younger tillers during seed development. It is therefore hypothesized that a proportion of the 40 - 60 mg of stem dry weight lost, which was unaccounted for in the seed dry weight, was remobilisted into the release of new tillers, thus maintaining the perennial nature of the species. There was a general trend for increasing Moddus rate to reduce the number of stems with daughter tillers (Table 5.18) however all treatments lost the same amount of weight from the maximum gained post anthesis. It is possible that the pattern of where assimilate ended up was different across Moddus treatments. All stems lost about 80 mg of weight which they had gained post anthesis. In crops which lodged this could have ended up as regrowth tillers compared with the high Moddus treatments where stems produced approximately 70 mg more seed than their untreated counterparts.

These results demonstrate that the use of Moddus can delay vegetative regrowth through a reduction in stem length and lodging. Therefore suppression of daughter tillers occurs because the canopy integrity is maintained. Once lodging occurs the ratio of red:far red light at the base of the canopy increases which would then encourage vegetative development. If plant breeders could reduce the stem length and/or the susceptibility to lodging the release of tillers may be reduced. However this does not remove the apparent trait of storing carbohydrate in the lower stem, presumably to maintain the perennial growth habit by the release of new tillers. Because crop persistence is a major issue for the end user (pastures/turf), it is hard to see that plant breeders would consider selecting cultivars where carbohydrate is stored higher in the stem.

The results of this study suggest perennial ryegrass has the ability to fill available seed and accumulate assimilate in the stem concurrently. The final harvest index of main stems was high at between 21 and 44% for individual treatments. The change in harvest index per day (dHI/dt) and per °C day was linear (Figure 5.5; Figure 5.6). A linear change in HI with time has been shown for a number of crop species including maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L) (Muchow, 1988) and wheat (Amir and Sinclair, 1991; Moot *et al*., 1996) (refer to section 2.3.11 for further detail).

However, in this study there was a cultivar by sowing date interaction and Moddus effect on dHI/dt when analysed by thermal time (Table 5.20). The use of Moddus increased the dHI/dt when expressed in both days and thermal time. The 1600 ml/ha rate of Moddus increased the rate of dHI/dt from 0.043 to 0.053%/°C day (Table 5.20) or from 0.68 to 0.85%/day (Table 5.19, Table 5.21). The calculated values in this study are lower than those for wheat at 0.90 – 1.19%/day (Moot *et al*., 1996).

The dHI/dt for individual seed heads was linear following a short lag phase of 60 °C days after anthesis . The lag phase is shorter when analysed by dHI/dt analysis compared with the seed growth data at 140 °C days. The difference in the calculated lag phase for dHI/dt is associated with the inclusion of the data from the lag phase in the regression analysis (Figure 5.5) however, the fit of the line to the data makes no suggestion that it should be removed (e.g. $R^2$ values above 96%, see Appendix B.6). Therefore, the spread in anthesis within the ear has influenced the calculation of the lag phase. Following the lag phase, stem weight increased in all treatments until at least 310 °C days. During this time frame seed undergoes a rapid increase in cell number (lag phase) followed by the majority of seed filling (dry weight increase). During the period of the lag phase the seed has limited requirement for assimilate, which is therefore partitioned into the stem, possibly for later partitioning to the seed. Later in seed filling when stem weight began to decrease, seed filling rate relative to individual stem biomass was maintained. If the dHI/dt is constant higher biomass at anthesis should give higher harvested yield. However, on main stems this was not the case as the 0

Moddus stems were heaviest at anthesis (Table 5.11) and showed the lowest yield. No other data on dHI/dt for grasses was found to compare this result with. In the analysis on wheat by Moot *et al*. (1996) lodging was shown to decrease the rate of dHI/dt in some crops. Therefore in crops of perennial ryegrass where lodging is common but the timing of the onset of lodging influenced by management a single figure for dHI/dt may be difficult to establish.

The application of Moddus increased the final harvest index and the rate of dHI/dt while creating shorter stems which were heavier than the untreated plots. This implies that shorter stems either transferred more assimilates at a faster rate to developing seeds or had less ability to store assimilate. It is generally accepted that shorter stems, as a result of having shorter internodes, have less capacity to store assimilate (Hay and Walker 1989). Therefore a shorter stem should stop competing with developing seed earlier if the storage capacity of the stem in reached (Bonnett and Incoll 1992).

### 5.11.1 Conclusions

- Seed growth in perennial ryegrass was described by a sigmoid growth curve and should be expressed as a function of thermal time with a base temperature of 0°C.
- The lag phase (150 °C days) of seed growth was longer than modern wheat cultivars while the duration of linear seed growth (294°C days) was short compared with cereals.
- Changes in final seed yield were a result of increased rate of seed filling per head.
- Stem dry weight continued to increase following anthesis until approximately 75% seed filling.
- The stem acts as a storage organ for assimilate for between 300 and 400 °C days (net sink) when the seed sink becomes large enough to attract assimilate from the stem. However the seed sink was insufficient to capture all assimilate from the stem and some remained after seed maturity.
- Harvest index for mainstems was high relative to whole plots. The dHI/dt was linear but affected by Moddus and sowing date treatments which were implicated in lodging.
- Seed yield of perennial ryegrass could be improved by growers and plant breeders selecting cultivars with shorter stems that are likely to compete with the developing seed sink for a shorter time period.

# Chapter 6

# General Discussion

The overall aim of this experiment was to investigate parameters required to describe perennial ryegrass seed growth. A quantitative description of these then allows the development of simulation models and decision support systems that could be used to examine factors associated with increased seed yield. To generate variability in seed growth additional agronomic treatments of sowing date and Moddus application were used. These were expected to produce a wide range of seed growth responses that could be examined to produce unifying relationships. At the same time the agronomic responses of seed yield were obtained. It is hoped that improved understanding of the processes that occur post anthesis could guide plant breeders to find selection traits that increase seed yield. Currently growers apply Moddus to perennial ryegrass seed crops due to results from agronomic trials without any physiological basis to explain why seed yield increases. This knowledge of the physiology underpinning how Moddus increases seed yield could lead to further increases in seed yield and further desirable traits for the use in plant breeding.

The seed yields shown in this experiment are high by international standards with 3000 kg/ha achieved in 'Meridian' and 'Bronsyn'. However, the individual treatment range was 1300 – 3350 kg/ha which gives the basis for discussion.

### 6.1.1 Phases of seed growth

Analysis of the duration of seed growth showed that 'Meridian', 'Bronsyn' and 'Grasslands Impact' had similar durations of seed growth. Following peak anthesis, the initial lag phase of 150 °C days preceded a linear phase of seed growth lasting about 294 °C days. The duration of seed growth (time to 95% of final seed weight) was constant in thermal time between these three cultivars of perennial ryegrass at 443°C days. Therefore it seems unlikely that growers and industry are able to influence the time from anthesis to maturity unless they can control canopy temperature. The use of irrigation may extend the duration of the seed filling period in days if the canopy can access water for evaporative cooling (Jones *et al*., 2009). However, seasonal variation in temperature and rainfall will lead to greater variation in the number of days from anthesis to maturity. The lag phase was quantified as 50 °C days longer than that shown for more modern wheat cultivars by Loss *et al*. (1989). In wheat, the lag phase has been reduced from approximately 190 °C days in older cultivars to approximately 100 °C days in more modern cultivars, thus extending the linear phase of seed growth. This suggests the physiological factors that contribute to the lag phase are under some genetic control and therefore offer some potential for selection.

The duration of seed fill was constant in thermal time at 443 °C days, thus management that increases the rate of seed filling is required to maximise yield. The application of Moddus created a canopy structure that increased the rate of seed filling from 0.50 to 0.74 mg/°C day/head for 0 and 1600 ml/ha respectively (Table 5.10). Not surprisingly the plot seed yields were also increased by these applications.

### Seed growth phase implications for breeders

The selection for a shorter lag phase could produce more seeds/m$^2$ due to an increase in the time spent during the linear phase of growth. While selection for reduced lag phase has the potential to reduce seed size thought the number of cells produced, increasing the duration of the linear phase of seed growth should see the conversion of more under size seed into first grade seed. In this experiment 'Grasslands Impact' converted less seeds to first grade seed compared with 'Bronsyn' and 'Meridian' (73 vs 85%) with a trend for a reduced rate of seed filling being the main contributor. If the duration of filling was extended in 'Grasslands Impact' then seed yields similar to other cultivars may be achievable. Currently there are many new cultivars which have similar 'Spanish' genetics and these also have lower seed yields.

There was also a trend for cultivar (and therefore genetics) to affect the rate of seed filling which requires further investigation to determine if genetic variation exists. If so it may lead to large advances in seed yield as the main difference in seed yield was through an increase in the rate of seed filling during the linear phase of seed growth. While the duration of seed growth remained constant.

### Seed growth phase implications for seed producers

Seed producers have no control over the phases of growth which are set in thermal time without changing the climatic conditions experienced by the crop. This is possible through a change in location or anthesis date, both which may alter the temperature experienced post anthesis. Since heading date is genetically controlled through interactions of day length and temperature, some form of chemical or lighting treatment would be required to alter heading date if seed crops were grown at the same location. However, growers can implement stem shorting to increase the rate of seed filling per head. In the agronomic experiment the application of Moddus at 1600 ml/ha increased seed yield by 58% compared with the untreated control. This was attributed to the production of a greater number of seeds/m$^2$. For the individual stems, the rate of seed filling increased from 0.50 to 0.74 mg/°C day/head when 1600 ml/ha Moddus was applied. Similar results could be expected by growers.

### 6.1.2 Stem/seed interactions

The large seed yield increase reported from the application of Moddus (a 58% increase at 1600 ml/ha) was predominantly due to stem shorting and an increase in seeds/spikelet. Following anthesis stems continued to increase in dry weight to a maximum at about 75% of final seed weight. Stems acted as a net sink for assimilate until late seed filling, (310 - 400 °C days after peak anthesis) when the seed sink was sufficient to attract assimilate for the final 50 – 100 °C days. Thus, from pollination, during the lag phase and during early seed growth the stem competed directly for assimilate with the growing seed. During early seed growth it appears the seed can source its own assimilate without input from the stem as a linear growth rate was maintained. Reducing the stem length by 0.15 m increased the rate of seed filling by 0.24 mg/°C days/head. This suggests that the stem storage space was reduced and it was a weaker sink, so more assimilate was available for transport to the developing seed. This is a similar response to the addition of the $Rht_2$ dwarfing gene in wheat (Austin *et al*. 1980). The results shown here from the application of Moddus, i.e. reduced stem length, increased harvest index, and increased seed/$m^2$, are similar to the advances that have been made in wheat over approximately 100 years of intensive breeding (Austin *et al*. 1980). Anslow (1964) showed seed weight decreased from the basal to distal spikelets and from basal to distal seeds within spikelets. Therefore seeds located further from the source of assimilate (stem) appeared to be lighter. Seeds from individual spikelets were not separated in this study but Moddus shortened the rachis length which may increase the ability for the distal spikelets to attract assimilate (Austin *et al*., 1980).

#### Stem/seed interactions and implications for breeders

The production of a stem which has a smaller sink capacity may significantly increase seed production. However, any selection criteria that could reduce persistence would be detrimental to the perennial ryegrass end user. It is likely the increased stem weight following anthesis supplies carbohydrate for regrowth following seed maturity and is therefore a critical component in maintenance of the perennial growth habit. To date there has been no dwarfing gene identified for perennial ryegrass (Boelt and Studer, 2010). However, genetic variation is likely to exist and therefore the selection for shorter stems may simulate the response which has been achieved in this experiment by the use of Moddus. In this experiment there was a trend for ‘Meridian’ and ‘Bronsyn’ to have a higher rate of seed filling per head compared with ‘Grasslands Impact’ when stem length was similar. This indicates genetic variability exists within perennial ryegrass for either stem capacity or the ability to transport assimilate to the seed head.

**Stem/seed interactions and implications for seed producers**

Growers can currently achieve reduced stem and rachis length through the application of Moddus. However, many countries impose restrictions on the use of agrichemicals for environmental reasons. Therefore advances from plant breeders are likely to increase in importance in the long term. If growers could alter the timing of when assimilate accumulated in the stem i.e. fill the stem to capacity earlier, then increases in seed yield may be possible due to reduced competition during seed filling. Options may include either reducing the stems capacity e.g. higher rates of Moddus and reducing stem length further, or through increasing the production of assimilate through maintaining green leaf and stem area with fungicides. A reduction in vegetative tillers through reduced lodging will reduce competition from non-seed producing leaves for solar radiation and water.

### 6.1.3 Harvest index

The harvest index of perennial ryegrass is low and variable. In whole plots the range was 10.8 – 25.6% while on main stems the range was 25 – 40% (Figure 5.5). The dHI/dt was constant in thermal time suggesting that seed yield growth could be modelled if the increase in biomass was known. In this study Moddus increased the dHI/dt by 0.01%/°C day when applied at 1600 ml/ha compared with 0 ml/ha. The 1600 ml/ha Moddus rate also reduced lodging. In wheat dHI/dt is stable across a wide range of environments and management inputs, with the exception of lodging which was shown to reduce dHI/dt (Moot *et al*., 1996). Lodging is common in perennial ryegrass seed crops, however the extent of lodging differs considerably which may make the modelling of perennial ryegrass difficult. However, a value of 0.05%/°C day (Table 5.19, Table 5.20) may be suitable for farm scale crops in New Zealand as most growers apply greater than 1000 ml/ha Moddus. The use 0.05%/head/°C day requires further investigation as these results are for main stems only. In perennial ryegrass seed crops there is a cross section of tiller age. The sub tillers of the main stem are likely to have a lower rate of seed filling and therefore potentially a lower dHI/dt. However these tillers are also likely to be lighter, thus possibly offsetting the lower seed weight.

Growers can influence the HI at harvest through defoliation leading to reductions in biomass during spring, prior to stem extension and the application of Moddus. However, they currently have very few other options. Screening for harvest index in breeding programmes would be a relatively simple process at harvest and could lead to long term gains. Growers and plant breeders are unlikely to investigate dHI/dt unless stem shorting and a reduction in lodging occurs first. However, for those interested in modelling seed yield in perennial ryegrass e.g. water and nutrient use efficiency the dHI/dt is worth further investigation.

Screening for seed production is a component of most ryegrass breeding programs and is the final selection operation prior to commercialisation. As such is in some scenarios it is a case of selecting the best from a bad bunch to meet the demands of the end-user, either for forage production or sports fields. However, this research has identified some simple traits which could be assessed in each seed production cycle, including stem length, lodging susceptibility and harvest index. The overall challenge for breeders, physiologists and growers remains to increase the number of seeds/$m^2$ which reach a saleable weight. Of the options discussed stem length appears the simplest to implement.

This study has contributed to the understanding of how the straw shortener 'Moddus' increases seed yield in perennial ryegrass seed crops and makes suggestions where future genetic advances could be made. In forage type perennial ryegrass, it is currently seed producers who have the largest area of influence on seed production through the application of Moddus. The higher seed yields of 'Meridian' and 'Bronsyn' suggest some genetic gains compared with 'Grasslands Impact'. However, selections for shorter reproductive stem length and greater resistance to lodging are required to increase the potential seed yield in perennial ryegrass.

**Conclusions**

- Seed growth follows a sigmiod pattern and was more accurately described by thermal time (temperature base 0 °C) following anthesis compared with days after anthesis.
- The lag phase, 150 °C days, and the duration of the linear phase, 294 °C days, were constant between all treatments. Seed yield differences were a result of changes in the slope of the linear phase of seed filling.
- Moddus produced significant increases in seed yield through changes in the rate of seed filling per stem. Moddus was associated with decreasing stem length and reduced lodging.
- Stems increased in weight following anthesis until 310 - 400 °C days mid seed fill, indicating a competing sink for resources until approximately 75% seed filling.
- Shorter stems had a higher harvest index, increased rate of seed filling, higher seed yield and less lodging at harvest.
- The increase in harvest index per day was linear following a short lag phase after anthesis.
- Further investigations should attempt to determine if stem reserves can be further depleted in perennial ryegrass

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# Appendix A

## Yield components

**Table A.1.** Yield components from quadrats for three cultivars of perennial ryegrass sown on two dates and treated with three rates of Moddus at Lincoln University during the 2008/09 growing season.

| Sowing date | Cultivar | Moddus (ml/ha) | Heads/m$^2$ | spk/head | spk/m$^2$ | seeds/spk | seeds/m$^2$ | TSW |
|---|---|---|---|---|---|---|---|---|
| 1 April 2008 | Meridian | 0 | 2216 | 21.6 | 47869 | 1.11 | 53274 | 2.50 |
| | | 800 | 2259 | 21.4 | 48422 | 1.54 | 74601 | 2.45 |
| | | 1600 | 2090 | 20.9 | 43656 | 2.25 | 98107 | 2.53 |
| | | | **2189** | **21.3** | **46649** | **1.64** | **75328** | **2.50** |
| | Bronsyn | 0 | 2106 | 22.5 | 47325 | 1.21 | 58032 | 2.58 |
| | | 800 | 2282 | 22.2 | 50841 | 1.65 | 84655 | 2.51 |
| | | 1600 | 2213 | 22.3 | 49447 | 2.20 | 108595 | 2.46 |
| | | | **2200** | **22.3** | **49204** | **1.68** | **83761** | **2.51** |
| | Impact | 0 | 2340 | 21.2 | 49451 | 1.62 | 79692 | 1.94 |
| | | 800 | 2349 | 22.1 | 51945 | 1.63 | 83876 | 2.03 |
| | | 1600 | 2394 | 21.5 | 51415 | 2.34 | 119483 | 1.92 |
| | | | **2361** | **21.6** | **50937** | **1.86** | **94350** | **1.96** |
| | **1 April mean** | | **2250** | **21.7** | **48930** | **1.73** | **84480** | **2.32** |
| 14 May 2008 | Meridian | 0 | 2497 | 20.1 | 50117 | 1.44 | 71687 | 2.87 |
| | | 800 | 2298 | 21.1 | 48551 | 2.24 | 105901 | 2.64 |
| | | 1600 | 2280 | 19.8 | 45080 | 2.40 | 108046 | 2.83 |
| | | | **2358** | **20.3** | **47916** | **2.03** | **95212** | **2.78** |
| | Bronsyn | 0 | 2354 | 19.1 | 45363 | 1.90 | 81615 | 2.63 |
| | | 800 | 2268 | 20.7 | 46874 | 2.29 | 107768 | 2.53 |
| | | 1600 | 2275 | 19.7 | 44587 | 2.82 | 125504 | 2.70 |
| | | | **2299** | **19.8** | **45608** | **2.34** | **104962** | **2.62** |
| | Impact | 0 | 2269 | 21.4 | 48656 | 1.69 | 81853 | 2.12 |
| | | 800 | 2332 | 23.8 | 55457 | 1.67 | 92782 | 2.14 |
| | | 1600 | 2479 | 20.5 | 50809 | 2.39 | 121822 | 2.04 |
| | | | **2360** | **21.9** | **51641** | **1.92** | **98819** | **2.10** |
| | **14 May mean** | | **2339** | **20.7** | **48388** | **2.09** | **99664** | **2.50** |
| | **Grand Mean** | | **2295** | **21.2** | **48659** | **1.91** | **92072** | **2.41** |
| P value - Sowing date | | | 0.135 | 0.199 | 0.806 | - | - | - |
| P value - Cultivar | | | 0.107 | 0.222 | 0.110 | - | - | - |
| P value – Moddus rate | | | 0.993 | 0.070 | 0.395 | <0.01 | <0.001 | 0.382 |
| $LSD_{0.05}$ - Sowing date | | | NS | NS | NS | - | - | - |
| $LSD_{0.05}$ - Cultivar | | | NS | NS | NS | - | - | - |
| $LSD_{0.05}$ – Moddus rate | | | NS | NS | NS | 0.205 | 9136 | NS |
| Sowing date*Cultivar | | | | | | 0.028 | | 0.022 |
| | | | $LSD_{0.05}$ - within sowing date | | | 0.288 | | 0.087 |
| | | | $LSD_{0.05}$ - between sowing date | | | 0.524 | | 0.084 |

## Change in harvest index per day

**Table A. 2** Regression coefficients for the full interaction data for change in harvest index per day for three cultivars or perennial ryegrass (*Lolium perenne*) with three Moddus rates (a.i. 250 g/l Trinexapac ethyl) when sown either 1 April or 14 May, Lincoln University, Canterbury, 2008/09 growing season.

| **Sowing date** | **Cultivar** | **Moddus (ml/ha)** | **Slope** | **Constant (y-axis intercept)** | **x-axis intercept (days)** |
|---|---|---|---|---|---|
| 1 April | Meridian | 0 | 0.51 | -2.07 | 3.91 |
| | | 800 | 0.52 | -1.92 | 3.70 |
| | | 1600 | 0.55 | -1.72 | 3.51 |
| | | | **0.53** | **-1.90** | **3.70** |
| | Bronsyn | 0 | 0.71 | -2.60 | 3.54 |
| | | 800 | 0.70 | -3.44 | 4.76 |
| | | 1600 | 0.79 | -2.99 | 3.71 |
| | | | **0.73** | **-3.01** | **4.01** |
| | Impact | 0 | 0.46 | -1.61 | 2.68 |
| | | 800 | 0.69 | -1.88 | 2.65 |
| | | 1600 | 0.70 | -2.76 | 3.27 |
| | | | **0.62** | **-2.08** | **2.86** |
| 1 April means | | | **0.63** | **-2.33** | **3.53** |
| 14 May | Meridian | 0 | 0.82 | -4.89 | 6.16 |
| | | 800 | 0.97 | -5.84 | 5.80 |
| | | 1600 | 1.01 | -5.47 | 5.15 |
| | | | **0.93** | **-5.40** | **5.71** |
| | Bronsyn | 0 | 0.87 | -4.13 | 4.55 |
| | | 800 | 0.88 | -4.35 | 4.72 |
| | | 1600 | 1.10 | -7.79 | 7.02 |
| | | | **0.95** | **-5.43** | **5.43** |
| | Impact | 0 | 0.72 | -0.93 | 1.48 |
| | | 800 | 0.81 | -4.47 | 5.51 |
| | | 1600 | 0.92 | -5.15 | 5.70 |
| | | | **0.82** | **-3.51** | **4.23** |
| 14 May means | | | 0.90 | -4.78 | 5.12 |
| P value - Sowing date | | | 0.012 | 0.059 | 0.078 |
| P value - Cultivar | | | 0.062 | 0.423 | 0.540 |
| P value – Moddus rate | | | 0.015 | 0.066 | 0.369 |
| $LSD_{0.05}$ - Sowing date | | | 0.133 | NS | NS |
| $LSD_{0.05}$ - Cultivar | | | NS | NS | NS |
| $LSD_{0.05}$ – Moddus rate | | | 0.107 | NS | NS |

**Note**. No significant (P<0.05) interactions

**Table A.3** Regression coefficients for the full interaction data for change in harvest index per day for three cultivars or perennial ryegrass (*Lolium perenne*) with three Moddus rates (a.i. 250 g/l Trinexapac ethyl) when sown either 1 April or 14 May, Lincoln University, Canterbury, 2008/09 growing season.

| Sowing date | Cultivar | Moddus (ml/ha) | Slope | Constant (y-axis intercept) | x-axis intercept (days) |
|---|---|---|---|---|---|
| 1 April | Meridian | 0 | 0.035 | -1.35 | 36.9 |
| | | 800 | 0.035 | -1.29 | 36.2 |
| | | 1600 | 0.038 | -0.84 | 25.6 |
| | | | **0.036** | **-1.16** | **32.9** |
| | Bronsyn | 0 | 0.046 | -2.24 | 49.0 |
| | | 800 | 0.047 | -3.38 | 102.5 |
| | | 1600 | 0.057 | -3.49 | 94.0 |
| | | | **0.050** | **-3.04** | **81.9** |
| | Impact | 0 | 0.027 | -0.90 | 24.4 |
| | | 800 | 0.038 | -0.72 | 18.2 |
| | | 1600 | 0.040 | -1.78 | 32.3 |
| | | | **0.035** | **-1.13** | **25.0** |
| | 1 April means | | **0.040** | **-1.78** | **46.6** |
| 14 May | Meridian | 0 | 0.055 | -5.12 | 96.2 |
| | | 800 | 0.065 | -6.09 | 90.8 |
| | | 1600 | 0.068 | -5.72 | 80.8 |
| | | | **0.062** | **-5.64** | **89.3** |
| | Bronsyn | 0 | 0.055 | -2.54 | 47.3 |
| | | 800 | 0.053 | -3.21 | 56.4 |
| | | 1600 | 0.068 | -6.77 | 64.1 |
| | | | **0.059** | **-4.17** | **56.0** |
| | Impact | 0 | 0.040 | -0.12 | 2.8 |
| | | 800 | 0.045 | -3.56 | 81.0 |
| | | 1600 | 0.050 | -3.77 | 76.4 |
| | | | **0.045** | **-2.48** | **53.4** |
| | 14 May means | | **0.055** | **-4.10** | **66.2** |
| | P value - Sowing date | | - | 0.082 | 0.367 |
| | P value - Cultivar | | - | 0.169 | 0.224 |
| | P value – Moddus rate | | 0.005 | 0.037 | 0.144 |
| | $LSD_{0.05}$ - Sowing date | | - | NS | NS |
| | $LSD_{0.05}$ - Cultivar | | - | NS | NS |
| | $LSD_{0.05}$ – Moddus rate | | 0.005 | 1.27 | NS |
| | Sowing date*cultivar | | 0.019 | 0.193 | 0.088 |
| | $LSD_{0.05}$ - within sowing date | | 0.0088 | NS | NS |
| | $LSD_{0.05}$ - between sowing date | | 0.0097 | NS | NS |

**Note**. There were no other significant (P<0.05) interactions

## Seed yield fractions

**Table A. 4.** Seed yield fractions for three cultivars of perennial ryegrass sown on two dates and treated with three rates of Moddus at Lincoln University in the 2008/09 growing season.

| Sowing date | Cultivar | Moddus rate (ml/ha) | First grade seed (kg/ha)* | Second grade seed (kg/ha)* | Total seed produced (kg/ha)* |
|---|---|---|---|---|---|
| 1 April 2008 | Meridian | 0 | 1324 | 287 | 1611 |
| | | 800 | 1833 | 289 | 2122 |
| | | 1600 | 2474 | 459 | 2933 |
| | | | **1877** | **345** | **2222** |
| | Bronsyn | 0 | 1487 | 441 | 1928 |
| | | 800 | 2136 | 459 | 2595 |
| | | 1600 | 2665 | 427 | 3092 |
| | | | **2096** | **442** | **2538** |
| | Impact | 0 | 1536 | 569 | 2105 |
| | | 800 | 1701 | 834 | 2535 |
| | | 1600 | 2279 | 684 | 2963 |
| | | | **1839** | **696** | **2534** |
| 14 May 2008 | Meridian | 0 | 2059 | 301 | 2360 |
| | | 800 | 2794 | 419 | 3213 |
| | | 1600 | 3056 | 420 | 3476 |
| | | | **2636** | **380** | **3016** |
| | Bronsyn | 0 | 2152 | 202 | 2354 |
| | | 800 | 2720 | 448 | 3168 |
| | | 1600 | 3383 | 459 | 3842 |
| | | | **2752** | **370** | **3121** |
| | Impact | 0 | 1734 | 664 | 2398 |
| | | 800 | 1986 | 848 | 2834 |
| | | 1600 | 2476 | 708 | 3184 |
| | | | **2065** | **740** | **2805** |
| | Mean | | 2211 | 495 | 2706 |

| | Sowing date | Cultivar | Moddus |
|---|---|---|---|
| **First grade seed** | | | |
| P value | 0.0021 | 0.025 | 0.0000 |
| SEM | 17.68 | 97.23 | 67.05 |
| $LSD_{0.05}$ | 107.60 | 317.10 | 195.72 |
| **Second grade seed** | | | |
| P value | 0.9611 | 0.0004 | 0.0146 |
| SEM | 29.46 | 39.8 | 33.01 |
| $LSD_{0.05}$ | NS | 129.8 | 96.34 |
| **Total seed produced** | | | |
| P value | 0.0132 | 0.3060 | 0.0000 |
| SEM | 45.10 | 93.68 | 65.35 |
| $LSD_{0.05}$ | 274.43 | NS | 190.76 |

**Note**; there were no Significant (P<0.05) interactions

* First grade seed has an individual weight greater than 1.5 mg, second grade individual seed weight in between 0.5 and 1.5 mg, total seed is the combination of first and second grade seed.

# Appendix B

## Curve and regression parameters

**Table B.1.** Curve parameters derived from Genstat 14 for a 'Gaussian' curve used to describe anthesis curves for three perennial ryegrass cultivars grown at Lincoln University in the 2008/09 growing season.

| Formula | Y = A + B*PRNORMAL(X;M;S**2) | | |
|---|---|---|---|
| | Parameter | estimate | s.e. |
| | S | 3.855 | 0.242 |
| | M | 1.216 | 0.193 |
| | B | 979.9 | 70.2 |
| | A | 0.9 | 2.74 |
| | $R^2$ value | 96.8 | |

**Table B.2.** Linear regression parameters derived from Genstat 14 used to describe the onset and rate of lodging as days after ear emergence for three perennial ryegrass cultivars sown on two dates and treated with three rates of Moddus at Lincoln University in the 2008/09 growing season.

| Sowing date | Cultivar | Moddus (ml/ha) | Constant | S.E. | Slope | S.E | $R^2$ |
|---|---|---|---|---|---|---|---|
| 1 April 2008 | Meridian | 0 | -49.6 | 13.0 | 2.75 | 0.318 | 90.5 |
| | | 800 | -72.4 | 14.6 | 1.90 | 0.254 | 91.9 |
| | | 1600 | -90.9 | 14.5 | 1.82 | 0.241 | 92.9 |
| | Bronsyn | 0 | -1.73 | 7.39 | 2.07 | 0.325 | 88.8 |
| | | 800 | -25.5 | 5.76 | 1.50 | 0.142 | 95.0 |
| | | 1600 | -47.1 | 11.8 | 1.36 | 0.258 | 84.8 |
| | Impact | 0 | -17.3 | 6.35 | 2.59 | 0.194 | 96.1 |
| | | 800 | -21.7 | 4.11 | 1.76 | 0.125 | 96.4 |
| | | 1600 | -54.5 | 8.47 | 1.93 | 0.187 | 94.6 |
| 14 May 2008 | Meridian | 0 | -50.6 | 5.27 | 2.67 | 0.129 | 98.3 |
| | | 800 | -68.1 | 4.27 | 2.24 | 0.088 | 99.4 |
| | | 1600 | -60.2 | 3.38 | 1.35 | 0.059 | 99.4 |
| | Bronsyn | 0 | -22.8 | 3.86 | 2.17 | 0.107 | 98.3 |
| | | 800 | -39.7 | 11.1 | 1.30 | 0.258 | 86.4 |
| | | 1600 | -47.6 | 10.7 | 1.29 | 0.232 | 90.9 |
| | Impact | 0 | -14.6 | 3.89 | 2.42 | 0.144 | 97.8 |
| | | 800 | -14.8 | 2.16 | 1.22 | 0.056 | 98.2 |
| | | 1600 | -19.8 | 5.32 | 0.90 | 0.119 | 91.6 |

**Table B.3.** Curve parameters derived from Genstat 14 for a 'Logistic' curve used to describe seed growth in days after peak anthesis for three perennial ryegrass cultivars treated with three rates of Moddus grown at Lincoln University in the 2008/09 growing season.

| Cultivar | Moddus rate (ml/ha) | Fitted curve = A + C/(1 + EXP(-B*(X - M))) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Fitted values and standard errors | | | | | | |
| | | C | S.E. | B | S.E. | M | S.E. | $R^2$ |
| Meridian | 0 | 163 | 27.0 | 0.154 | 0.05 | 26.4 | 2.29 | 88.5 |
| | 800 | 200 | 18.7 | 0.200 | 0.06 | 27.3 | 1.49 | 89.4 |
| | 1600 | 217 | 12.2 | 0.203 | 0.03 | 27.0 | 0.903 | 95.8 |
| Bronsyn | 0 | 140 | 9.94 | 0.298 | 0.06 | 18.1 | 0.881 | 90.3 |
| | 800 | 149 | 13.1 | 0.245 | 0.05 | 19.0 | 1.06 | 94.3 |
| | 1600 | 195 | 19.4 | 0.266 | 0.03 | 18.8 | 1.05 | 97.4 |
| Grasslands Impact | 0 | 100 | 10.7 | 0.273 | 0.08 | 19.5 | 1.3 | 92 |
| | 800 | 163 | 19.3 | 0.244 | 0.04 | 20.4 | 1.35 | 90.5 |
| | 1600 | 181 | 14.3 | 0.269 | 0.04 | 21.6 | 0.906 | 96.6 |

Note. A = curve origin which was set to zero

**Table B.4.** Curve parameters derived from Genstat 14 for a 'Logistic' curve used to describe seed growth in thermal time after peak anthesis for three perennial ryegrass cultivars treated with three rates of Moddus, grown at Lincoln University in the 2008/09 growing season.

| Cultivar | Moddus rate (ml/ha) | Fitted curve = A + C/(1 + EXP(-B*(X - M))) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Fitted values and standard errors | | | | | | |
| | | C | S.E. | B | S.E. | M | S.E. | $R^2$ |
| Meridian | 0 | 165 | 19.0 | 0.011 | 0.002 | 311 | 19.6 | 95.4 |
| | 800 | 176 | 9.12 | 0.015 | 0.003 | 329 | 9.56 | 97.4 |
| | 1600 | 211 | 13.7 | 0.012 | 0.002 | 321 | 12.3 | 97.4 |
| Bronsyn | 0 | 138 | 11.1 | 0.017 | 0.004 | 288 | 14.8 | 93.8 |
| | 800 | 148 | 11.2 | 0.015 | 0.003 | 272 | 13.9 | 95.5 |
| | 1600 | 224 | 22.5 | 0.011 | 0.002 | 280 | 17.4 | 96.9 |
| Grasslands Impact | 0 | 111 | 15.2 | 0.011 | 0.003 | 267 | 27.1 | 93.4 |
| | 800 | 168 | 14.7 | 0.01 | 0.002 | 275 | 18.0 | 96.7 |
| | 1600 | 202 | 16.8 | 0.011 | 0.002 | 294 | 17.0 | 96.2 |

Note. A = curve origin which was set to zero

**Table B.5.** Curve parameters derived from Genstat 14 for a 'quadratic polynomial' curves used describe change in stem weight in thermal time following peak anthesis for three perennial ryegrass cultivars treated with three rates of Moddus and grown at Lincoln University in the 2008/09 growing season.

| Cultivar | Moddus rate (ml/ha) | Fitted curve = (Quad*$x^2$)+(Lin*x) + Constant | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Fitted values and standard errors | | | | | | |
| | | Constant | S.E. | Lin | S.E. | Quad | S.E. | $R^2$ |
| Meridian | 0 | -17.8 | 15.1 | 0.883 | 0.113 | -0.00121 | 0.00016 | 71.1 |
| | 800 | -3.2 | 15.1 | 0.891 | 0.110 | -0.00097 | 0.00016 | 80.9 |
| | 1600 | -27 | 15.4 | 0.847 | 0.112 | -0.00093 | 0.00017 | 77.8 |
| Bronsyn | 0 | 3.6 | 11.9 | 0.701 | 0.097 | -0.00112 | 0.00016 | 68.5 |
| | 800 | -3.2 | 14.9 | 0.830 | 0.125 | -0.00148 | 0.00022 | 76.1 |
| | 1600 | 6.7 | 16.0 | 0.883 | 0.134 | -0.00137 | 0.00023 | 65.7 |
| Grasslands Impact | 0 | 4.5 | 9.91 | 0.452 | 0.070 | -0.00071 | 0.00009 | 75.7 |
| | 800 | 6.0 | 10.4 | 0.449 | 0.071 | -0.00050 | 0.00009 | 67.1 |
| | 1600 | -17.3 | 11.5 | 0.623 | 0.082 | -0.00074 | 0.00011 | 72.7 |

**Table B.6.** Linear regression parameters derived from Genstat 14 used to describe the change in harvest index per degree C days for three cultivars of perennial ryegrass sown on two dates at Lincoln University in the 2008/09 growing season. .

| Fitted regression = ax+b | | | | | | |
|---|---|---|---|---|---|---|
| Sowing date | Cultivar | slope (a) | S.E. | constant (b) | S.E. | $R^2$ |
| 1 April 2008 | 'Meridian' | 0.036 | 0.001 | -1.16 | 0.571 | 98.0 |
| | 'Bronsyn' | 0.050 | 0.003 | -3.04 | 1.08 | 96.0 |
| | 'Grasslands Impact' | 0.035 | 0.002 | -1.13 | 0.814 | 96.7 |
| 14-May2008 | 'Meridian' | 0.063 | 0.004 | -5.64 | 1.60 | 95.2 |
| | 'Bronsyn' | 0.059 | 0.003 | -4.17 | 1.41 | 96.2 |
| | 'Grasslands Impact' | 0.050 | 0.002 | -2.48 | 0.853 | 97.8 |

**Table B.7.** Linear regression parameters derived from Genstat 14 used to describe the change in harvest index per day for perennial ryegrass sown on two dates and treated with three rates of Moddus at Lincoln University in the 2008/09 growing season.

| Fitted regression = ax+b | | | | | | |
|---|---|---|---|---|---|---|
| Sowing date | Moddus rate (ml/ha) | slope (a) | S.E. | constant (b) | S.E. | $R^2$ |
| 1 April 2008 | 0 | 0.561 | 0.025 | -2.09 | 0.696 | 97.1 |
| | 800 | 0.635 | 0.034 | -2.41 | 0.875 | 96.7 |
| | 1600 | 0.682 | 0.026 | -2.49 | 0.670 | 98.2 |
| 14-May2008 | 0 | 0.803 | 0.046 | -3.16 | 1.19 | 96.0 |
| | 800 | 0.888 | 0.059 | -4.89 | 1.51 | 95.4 |
| | 1600 | 1.01 | 0.063 | -6.14 | 1.63 | 95.6 |