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Seed yield and carbohydrate distribution in perennial ryegrass (*Lolium perenne L.*) and Italian ryegrass (*Lolium multiflorum* L.) seed crops

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A thesis

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by

Thomas Holmes

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Seed yield and carbohydrate distribution in perennial ryegrass (*Lolium perenne L.)* and Italian ryegrass (*Lolium multiflorum* L.) seed crops

by

Thomas Holmes

A field experiment was conducted to quantify the carbohydrate distribution in perennial (*Lolium perenne L.)* and Italian ryegrass (*Lolium multiflorum L.*) seed crops from anthesis to harvest. To do this, first year crops of diploid perennial ryegrass ('Samson') and a diploid Italian ryegrass cultivar ('Progrow') were sown on 14th May and 16th September 2015. A subsequent application of Moddus® (a.i. 250 g/l Trinexapac ethyl) plant growth regulator at three rates (0, 1,600 and 3,200 ml/ha) was applied at Zakoks growth stage 32.

Moddus® increased perennial and Italian ryegrass seed yield by approximately 29% from 1,600 ml/ha (1,651 kg/ha) to 3,200 ml/ha (2,134 kg/ha), with similar yields between 0 ml/ha (1,296 kg/ha) and 1,600 ml/ha. The seed yield increase in perennial and Italian ryegrass was achieved through increased number of seed produced per spikelets. The vegetative stem length in perennial and Italian ryegrass was reduced from 62 cm to 41 cm by application of 1,600 ml/ha of Moddus® and further reduced to 36 cm with 3,200 ml/ha.

The perennial ryegrass's stem component dry weight and water soluble carbohydrate (WSC) concentration were constant from anthesis through untill harvest. Despite Moddus® reducing the stem length and its storage capacity a large amount of WSC remained in the stem components. Perennial ryegrass had the capacity to fill and improve seed yield accumulating WSC simultaneously and converting this into starch up to harvest at 37 days after anthesis (DAA) with little or no remobilisation of WSC in the stem.

Moddus® decreased Italian ryegrass's stem components dry weight by 37% and WSC by 42% . The effect of Moddus® suggests the source and sink relationship between the stem and developing seed has been modified.The Italian ryegrass seed dry weight increased up to 7 DAA and declined until harvest with WSC and starch illustrating a similar pattern. When maximum seed weight, WSC and starch was achieved the stem component begun to decline. This demonstrates that Italian ryegrass stem may play a role in seed filling under conditions of limited assimilate supply with stem assimilate reserves remobilised to the developing seed.

Moddus® reduced overall lodging at the highest rate of 3,200 ml/ha and delayed the onset of lodging at the lower rate. Lodging progressed more quickly and had a greater effect on perennial ryegrass than Italian ryegrass. Lodging reduced perennial ryegrass seed yield with less WSC in the seed of control plants of 0 ml/ha (49.91 mg) compared to the highest Moddus® treatment of 3,200 ml/ha (59.63 mg). Italian ryegrass showed similar seed WSC between the control (88.6 mg) and the two Moddus® treatments of 1,600 ml/ha (94 mg) and 3,200 ml/ha (85.5 mg). This showed the degree which two ryegrass species seed sinks can compete for available assimilates. The relative lower seed depression in lodged Italian ryegrass compared to the perennial indicates a higher compensation potential. The Italian showed the ability to partition more assimilate reserves from the stem and leaves to support seed growth and development.

Acknowledgements

Table of Contents

List of Tables

List of Figures

1.   Introduction

In New Zealand, herbage seed production comprises approximately 35,000 ha of productive agricultural land annually (Pyke *et al.* 2004). Three main plant species, perennial ryegrass (*Lolium perenne L.),* white clover (*Trifolium repens L.)* and Italian ryegrass (*Lolium multiflorum* L.) dominate herbage seed production, accounting for greater than 85% of the annual herbage seed production area grown. The annual production of ryegrass seed crops ranges from 12,900 to 19,000 ha.

Perennial ryegrass plays an important role in New Zealand arable farmer's cropping rotations and by providing a steady return per ha, comprised of both grazing/silage and seed production, However seed production provides up to 85% of the return (Rolston & Archie 2005) . Perennial ryegrass seed yields have progressively increased at an average rate of 36 kg/ha/year with approximately 1,700 kg/ha produced in 2013 (Chynoweth *et al.* 2015). However, individual yields of over 3,000 kg/ha have been noted (Rolston *et al.* 2007).

Italian ryegrass plays a minor role in comparison to perennial ryegrass in seed production. The cultivars grown are mainly 'Grasslands Moata' and 'Tama' which also are predominantly used in New Zealand and thus do not produce large export volumes. Italian ryegrass seed yields have progressively increased at an average rate of 46 kg/ha/year with approximately 1,750 kg/ha produced in 2013 (Chynoweth *et al.* 2015).

The introduction of Moddus® in 1999 (a.i. 250 g/l Trinexapac ethyl) for lodging control gave seed growers a viable method for reducing lodging and the associated yield losses of up to 50% in both Italian and perennial ryegrass (Rolston *et al.* 2010, 2012). Recently, Tretheway and Rolston (2009) and Griffiths (2000) determined stem carbohydrates do not limit seed yield in non-lodged Italian and perennial ryegrass. Ryegrass seed yields are often low and variable with only 10-20% of the above ground matter harvested as seed. Seed yield is affected by the amount of carbohydrates transported to the seed (Elgersma 1990).

The objective of this study was to quantify both the water soluble carbohydrates (WSC) and starch changes from anthesis until harvest in both Italian and perennial ryegrass stem components (stem plus rachis) and the seed. To gain a better understanding of how carbohydrate assimilates within the different ryegrass plant components over time. Secondly, how these apparent carbohydrate changes influence both the Italian and perennial ryegrass harvest index's (HI) and associated yield components.

2.   Literature review

This review covers briefly how ryegrasses grow and develop with the main emphasis on anthesis until harvest. This includes a section on carbohydrate assimilate supply and the relationship between stem and seed post anthesis until harvest. The review concludes with a section on lodging and key pre-anthesis management.

2.1   Grass growth

In New Zealand's seed producing areas, perennial and Italian ryegrass have a wide sowing window as long as vernalisation requirements are met to ensure flowering. Early March is the recommended sowing time when soil temperatures are still 14°C. Moot *et al.* (2000) demonstrated that germination was optimal at 15°C with 99% germination for the perennial ryegrass cultivar 'Embassy'. Under lower temperatures of 5 and 10°C, germination was decreased and delayed. Italian ryegrass showed similar germination trends to perennial ryegrass requiring the same number of degree days (°Cd) of 90°Cd for 75% germination. However, Italian ryegrass required less degree days (145°Cd) to emerge than perennial ryegrass (160°Cd). Italian ryegrass demonstrated it was faster to emerge than perennial ryegrass,

Once germinated, ryegrass seed emerges producing a coleoptile through which the first leaf develops. Fine seminal roots emerge from the seed and frequently branch out compared to the first adventitious roots which appear later from stem nodes. Leaves arise from the shoot apex at the base of the tiller with subsequent leaf growth originating from meristem tissue. Only two leaves actively grow on each tiller apex at any one time (Langer 1979). The interval of time between the appearances of successive leaves is relatively constant during the vegetative development of a tiller. This is influenced by environmental factors such as temperature, photoperiod, and light quality. The critical leaf area is reached at the point where the plant canopy is capable of intercepting maximum solar radiation. The leaf reaches its final size, remaining on the plant for a certain period of time then begins to die. Senescence begins at the tip of the leaf and spreads downwards. The older and lower leaves often senesce at a rate near equal to the rate of new leaf appearance. So the number of leaves present on a tiller becomes relatively constant once leaf senescence begins to occur (Moore & Moser 1995).

By understanding the basis of how ryegrass growth and development changes over time, agronomists and growers can use these as guidelines for the timing of forage management practises. During ryegrass leaf development it is a useful time to apply management practises such as

defoliation, fertilisation, herbicide and plant growth regulators application (Moore *et al.* 1991). The decimal code scale developed for growth stages for cereals by Zadoks *et al.* (1974) has been the widely accepted growth scale used internationally for ryegrasses. The Zadoks scale is split into principal growth stages (**Table 2.1**) with the borderlines between the growth stages further classified into secondary growth stages.

Table 2.1: A decimal code for the growth stages of cereals principle growth stages (Zadoks *et al.* 1974).

1-digit code	Description
0	Germination
1	Seedling growth
2	Tillering
3	Stem elongation
4	Booting
5	Inflorescence emergence
6	Anthesis
7	Milk development
8	Dough development
9	Ripening

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 like characteristics that make it common amongst grazed pastures in New Zealand (Langer 1979). The production of tillers (tillering) is a continuous process in Italian and perennial ryegrass. The rate at which they emerge, grow and then die varies based on growing conditions and the management practices imposed on it. At any given time a grass plant can have a collection of tillers, all differing in age and size (Langer 1980). During seed production, a proportion of tillers remain vegetative, allowing the plant to regrow the following autumn. Tillering is vital for regeneration of the grass sward; it allows regrowth when the apical meristem is removed via cutting, grazing or inflorescence development. Furthermore, tillering allows for individual plants to establish and fill pasture gaps in the sward thus helping to maintain the longevity of the overall pasture sward.

Within a grass tiller, there is a basic repeating unit called a phytomer. Each phytomer consists of a node, internode, leaf blade and an axillary bud, each axillary bud is capable of producing a new tiller. Vegetative tillers have an active shoot meristem which can initiate the phytomer components with essentially no internode elongation. After reproductive development is initiated phytomer production ceases as the apex differentiates into inflorescence and the internode areas begin to extend. This period is termed elongation or the 'jointing' period.

The tiller begins to elongate usually in response to photoperiod, with the internode associated with the upper phytomers elongating in an acropetal (upwards) manner, while the lowest most internode remains basal (downwards) and not elongating. When maximum elongation occurs, the developing inflorescence exerts through the uppermost leaf sheath to form what is commonly called the 'boot stage' (Moore *et al.* 1991).

2.3 Reproductive initiation

The development towards anthesis and seed production in perennial ryegrass is driven by exposure to vernalisation, a period of cool temperature followed by a change in day length. In perennial ryegrass, the required temperature varies from 0 and 14°C, with the period of exposure depending on cultivar (Evans 1960). Once exposed to vernalisation, perennial ryegrass can respond to its specific photoperiod. Photoperiod is detected by a protein phytochrome located in the leaves of the grasses . Phytochrome's role is an biological clock, having the ability to change form during the day, providing a ratio for photoperiod detection.

Italian, as an annual, can flower without vernalisation if planted in spring. If planted in autumn Italian ryegrass's requirement for vernalisation is only minimal in comparison to perennial ryegrass which has an obligate vernalisation requirement (Heide 1994).

The first visual indicator of spikelet initiation is the appearance of the double ridge. The differentiation of each spikelet begins in the middle of the ear where the greatest elongation occurs, followed progressively with the remaining spikelets basipetally (downwards) and acropetally (upwards) developing, with the terminal spikelet the last to differentiate (Chynoweth 2012).

2.4 Pattern of anthesis

In both Italian and perennial ryegrass, anthesis begins proximally in the central spikelet and progresses basipetally and acropetally. In contrast to cereals, the spikelets of grasses closer to the tip

of the ear, flower earlier than the basal spikelets. Warringa *et al.* (1998) found that anthesis started in the proximal florets of the central florets that flowered first, and the upper spikelets in the ear flowered earlier than basel ones. Flowering proceeded within the spikelet from the base to the tip at a rate of 1.1-1.2 florets per day. The distal floret in the basal spikelet was the last to reach anthesis about 15 days after the onset of anthesis.

Seed ripening showed a similar trend to anthesis with upper spikelets ripening earlier than those of the basal spikelets. This was influenced by the rate of seed moisture loss ($P <0.01$) in the upper spikelets compared to the basal spikelets (Figure 2.1).
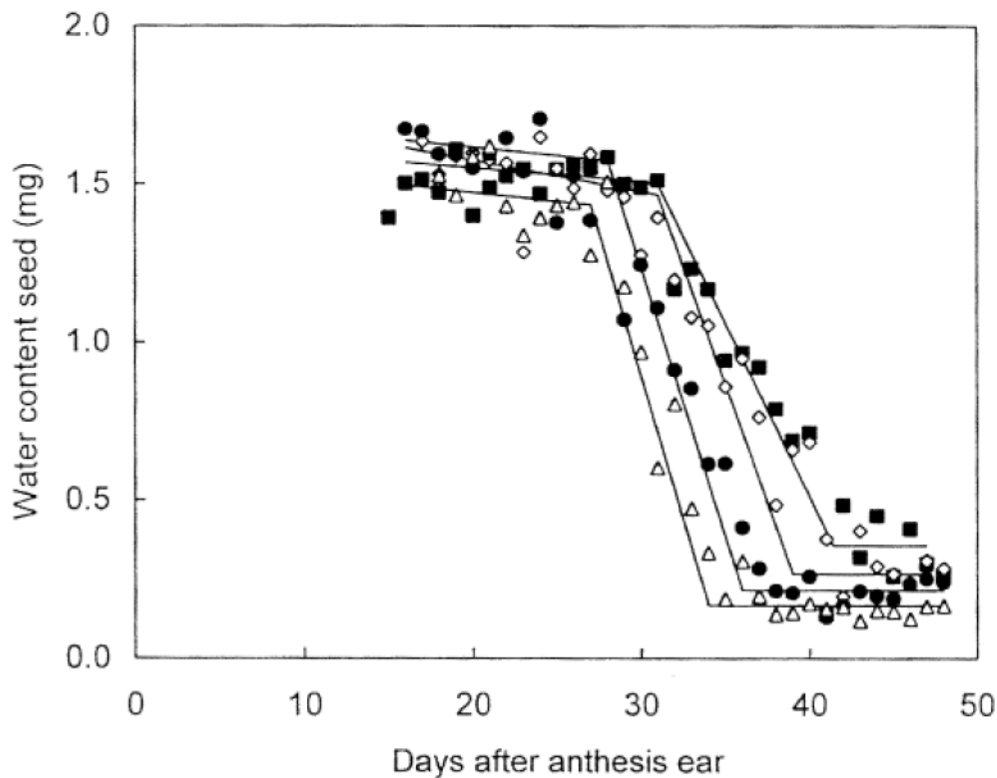
Figure 2.1: The changes in the average water content in the seeds of different spikelets with time after the onset of anthesis of the ear in *Lolium perenne*. Spikelets number 1 (basal) (■), 6 (◇), 10 (●) and 15 (△) were used. The ear had a total of 18 spikelets. Lines are the result of linear regression using a piecewise regression model (Warringa *et al.* 1998).

The average seed growth did not differ between spikelets, however, the growth duration (defined as the time between anthesis and maximum seed dry weight) increased from the upper spikelet (24.7 days) to the basel spikelet (30.2 days). On average within a spikelet, the water content of the central,

distal and proximal seed started to decline simultaneously, at a similar rate of 30 days after the onset of anthesis.

As a result of the flowering and ripening pattern, 4% of seed dry weight was accounted for between the spikelets and 89% was explained by the seed dry weights within the spikelets. Seed weight varied within the spikelet ranging from 1.86 mg in the proximal seed to 0.71 mg in the distal seed. The gradient in seed weights within the spikelet is primarily due to reduced growth rate and a lesser extent by the shorter growth duration. As the shorter duration is attributed to flowering time, the central and distal florets flower later than the proximal floret, but ripening occurs simultaneously.

As Warringa *et al.* (1998) express all attributes of seed growth in days after anthesis, it makes it difficult for a comparison to New Zealand conditions because of locational and seasonal differences. The maximum seed weight was 1.86 mg, being low with it expected in New Zealand to have an individual seed weight of 2 mg or a thousand seed weight of 2.0 g. Variation of results may be caused by variations in genotypes, location (environment) and management factors.

2.5   Source-sink relations

Understanding of plant physiology can be used in certain circumstances to determine if crop yield has been compromised through the capacity of the green tissue to assimilate (source limitation) or the capacity of harvested organs to store assimilates (sink limitation). Organs are considered either source or sinks when transport of assimilates is discussed. This depends on the direction of net assimilation. At a certain crop development stage, leaves become a net exporter of assimilate from current photosynthesis, however these can be stored for later remobilisation (Hay & Walker 1989). Until anthesis there are three main assimilation sinks; initiated leaves that are yet to be mature, developing stem and reserve accumulation in the stem or other tissues. After anthesis, the remaining leaves and green ear act as the source for the current photosynthate while the developing grain and stem are sinks. During the later stages of grain filling, assimilates from the stem are transported to the growing grains when photosynthetic tissue has undergone senescence (Hay & Porter 2006).

2.6   Sink and source relationship following anthesis

In *Lolium* species, the developing seed head appears to compete for assimilates with the elongating stem during the stem extension period. During stem extension, the stem uses assimilates to build structural components ultimately contributing to a mature seed head. Concurrently, the developing seed embryo is undergoing spikelet differentiation and building space for seed growth. Stems are a

strong sink during stem extension and rely on assimilates from the leaf tissue as there are not enough assimilates in the concentration required to sustain stem extension, possibly compromising the developing seed head. On the reproductive tiller, the younger, upper leaves supply carbohydrates to the upper stem and seed head, while the older, lower leaves mainly supply the lower stem, young tillers and root systems. After ear emergence, the seed head becomes the main source organ on the flowering tiller as its importance increases relatively as the leaves age (Clemence & Hebblethwaite 1984). Warringa and Marinissen (1997) support this, showing the source activity of leaves declined sharply, from 95% of total tiller photosynthesis to 16% at final harvest (Figure 2.2). This illustrates that the ear becomes the main assimilating organ on the flowering tiller as leaves are ageing.
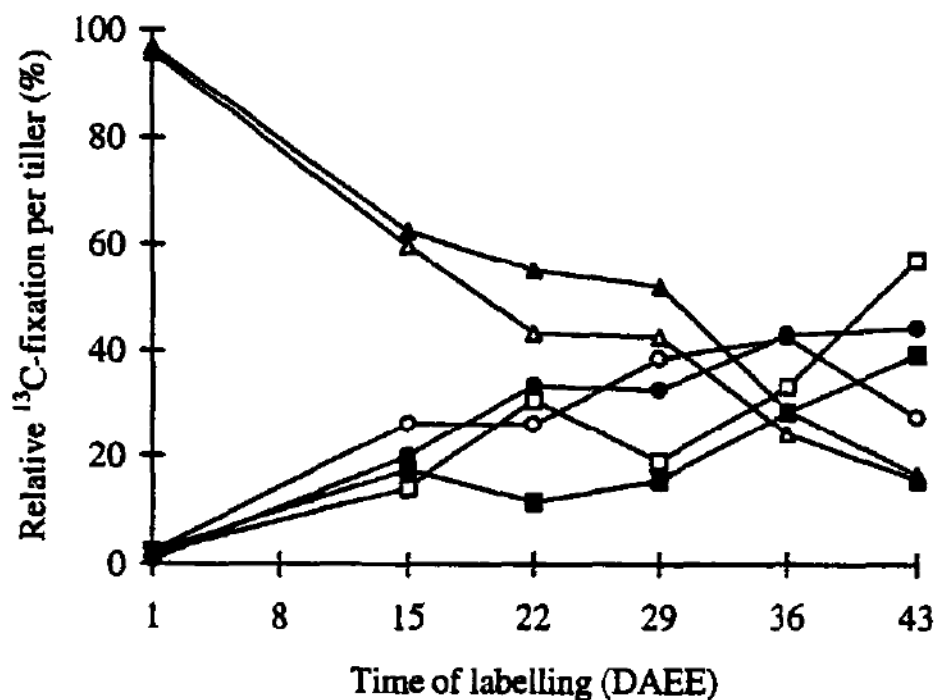
Figure 2.2: The relative fixation of ^{13}C-label by the ear, stem and leaves of the main tiller (open symbols) and the average group 2 tiller (closed symbols) in plants of *Lolium perenne* after labelling for 10 minutes. Time of labelling is indicated on the horizontal axis. (○,●) spikelet; (□,■) stem; (△,▲) leaves (Warringa & Marinissen 1997).

During anthesis the stem is a stronger sink than the seeds. Consequently carbon from the leaf activity are imported to the stems. Warringa and Marinissen (1997) showed that 70% of the ^{13}C-label fixed during anthesis remained in the stem at harvest. The stem remained a net importer of assimilate up to about mid seed filling, then ^{13}C-label was transported from stem and leaf tissue into developing

seeds. This is supported by Clemence and Hebblethwaite (1984) who also described the stem changing from a sink to source during the 'mid seed filling' period.

2.6.1 Carbohydrates

Forage grasses and cereals contain some of the most diverse structural and non-structural carbohydrates of all known plants. These structural WSC include low molecular weight (LMW) sucrose and high molecular weight (HMW) sucrose derived polymers of fructose (fructans). While the distribution of WSC is known during vegetative growth of grass, little work has been done on the mobilisation of WSC from vegetative organs to the seed during reproductive development. Tretheway and Rolston (2009) found total WSC concentration changed ($P <0.05$) from early head emergence through to harvest (Figure 2.3) with the concentration and ratio between LMW 'mobile' and HMW 'storage' WSC in the vegetative and reproductive tissues changing considerably during reproductive stages.
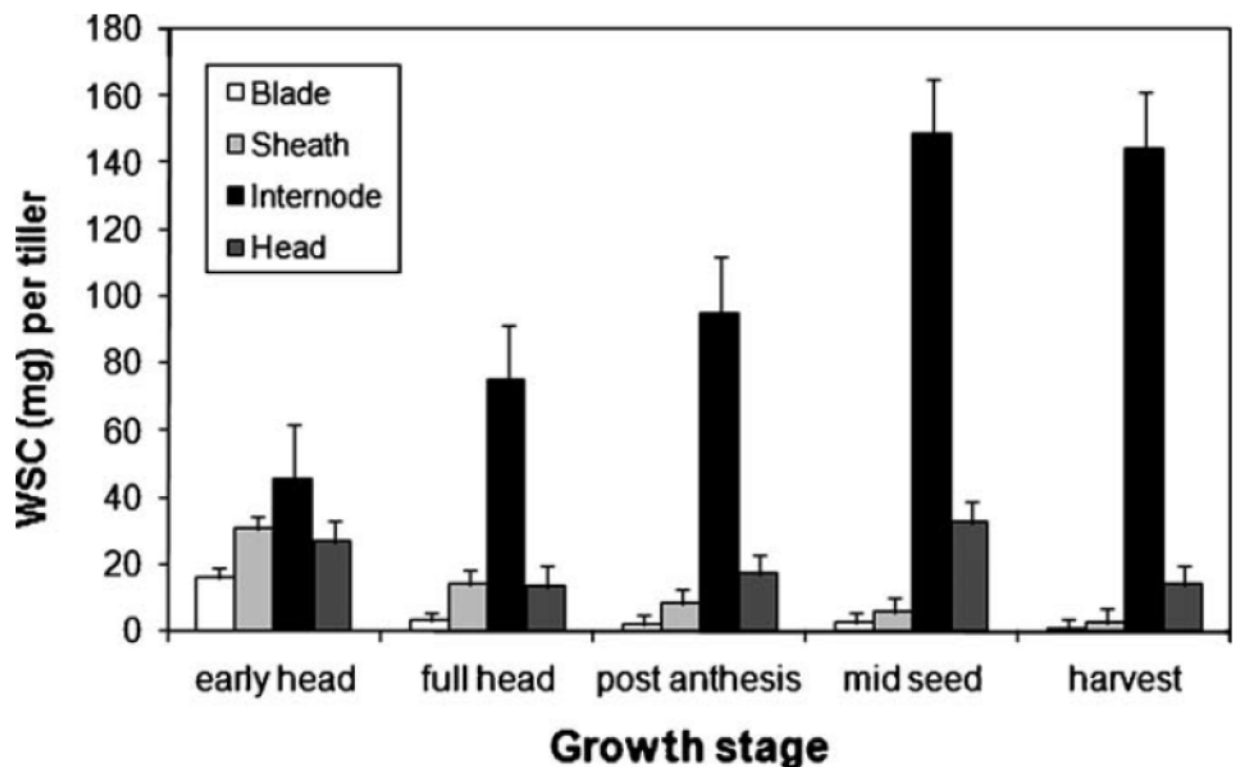
Figure 2.3: Total WSC of combined leaf blades, leaf sheaths, internodes and heads from early head emergence through to harvest. Bars=LSD (5%) (Tretheway & Rolston 2009).
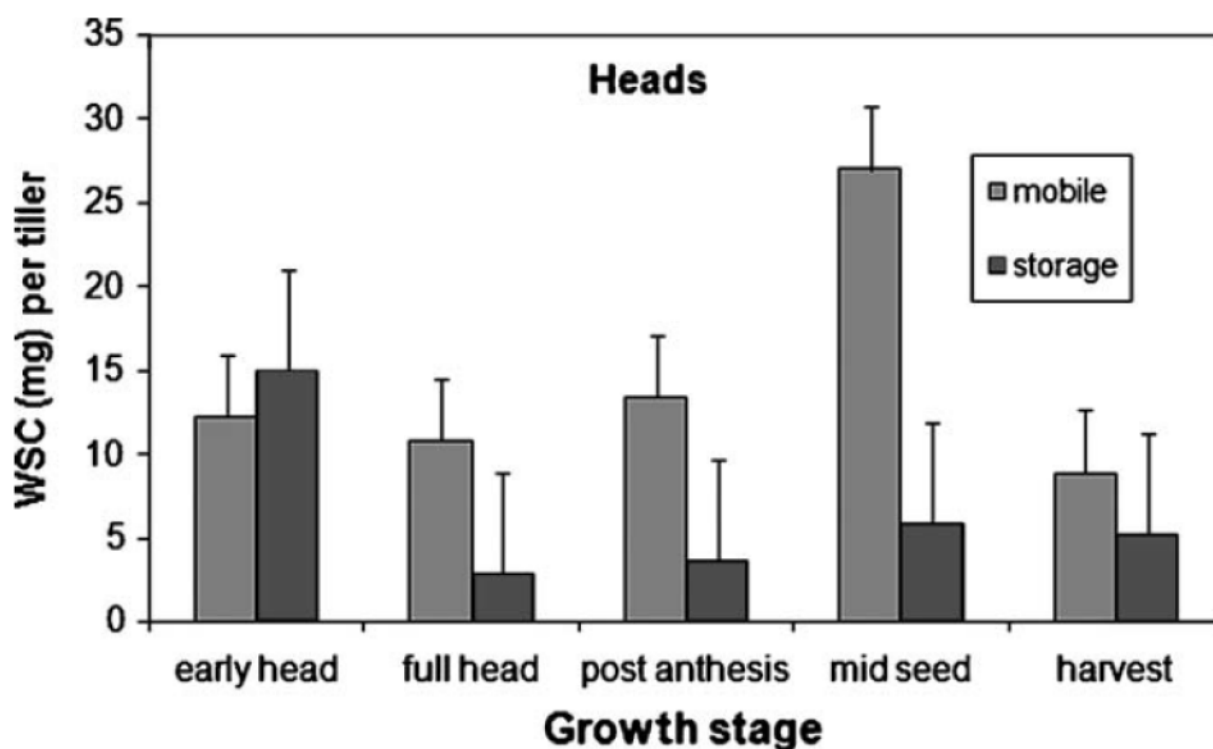
Figure 2.4: Concentration of 'mobile' and 'storage' WSC in heads of perennial ryegrass during reproductive development. Bars=LSD (%) (Tretheway & Rolston 2009).

In the reproductive head, in particular, the concentration of LMW WSC (P <0.05) increased between post-anthesis to mid-seed fill. In contrast HMW WSC (P <0.05) decreased from head emergence and remained low through to harvest (Figure 2.4). The internodes, in contrast to the reproductive heads, showed a significant increase in total concentration of HMW WSC from head emergence through to seed fill on to harvest. LMW WSC showed a decrease from full head emergence to post-anthesis and remained low through to harvest.

These results suggest that total WSC in vegetative tissues following anthesis does not limiting seed yield. During seed filling, a large amount of HMW 'storage' WSC accumulates in the basal and even the upper internodes. Warringa and Marinissen (1997) found in perennial ryegrass at harvest, WSC as a measure of total reducing sugars, accounted for 25% dry weight in the stem. While the total amount of assimilate in the tiller may not be limiting the seed yield, the distribution and remobilisation of available WSC to seed is seen to be an important factor limiting seed yield. This indicates the seed head itself may be an important factor driving seed fill.

Griffiths (2000) also found in both Italian and perennial ryegrass WSC generally increasing in all stem and inflorescence components from post anthesis to harvest in upright plants. In comparison the leaf

WSC declined. Further work by Griffiths (1992) found when source-sink relations were modified in Italian ryegrass by detillering and defoliation and / or detillering at anthesis the pattern of carbohydrate distribution of the stem changed. Through combined detillering and defoliation this reduced WSC in the lower and middle stem segments, with WSC not accumulating in the upper stem. A greater amount of carbohydrate partitioning occurred from the lower to upper stem. However, spike WSC remained low. In these conditions where carbohydrate levels were reduced, seed set was lowered. In contrast to the control plants, carbohydrate levels appeared adequate to support maximum seed set. These results suggest that under conditions where the source strength is limited, the stem plays a major role in partitioning assimilates to compensate for the sinks demand.

2.7   Seed development

The final stage in crop development is physiological maturity. In monocotyledonous species, including the grasses, seed development begins with fertilisation. Perennial ryegrass seed growth has been described previously by Hyde *et al.* (1959) and further examined by Warringa *et al.* (1998). There are three distinct phases in seed development described in detail for wheat by Loss *et al.* (1989).

Phase one is where rapid cell division occurs within the endosperm, with approximately 80% of total seed growth occurring. Due to the influx of water into the cells, this drives cell expansion, leading to exponential growth in whole seed fresh weight and water content. At this point the potential seed size is determined; this period is often referred to as the lag phase.

A period of linear weight gain follows with the synthesis and deposition of stored reserves. During this period, starch is deposited in the endosperm. The fresh weight remains relatively unchanged, while dry weight increases as water is displaced by accumulating storage reserves from storage tissues. The water content decline slows as the seed approaches maximum dry weight. At the point where maximum dry weight is achieved the seed is physiologically mature and is usually considered viable.

The final phase is where moisture is lost to the environment but the seed dry weight remains constant. When the seed reaches maximum dry weight and no more reserves accumulate, it is considered physiologically fully grown.

2.8 Harvest index and harvest loss

Perennial ryegrass harvest index (as seed percentage of above ground dry material) is only 10-20%, while the harvest index for most intensively cultivated small grain crops ranges from 40-60% (Hay 1995). Harvest index is a measure of the proportion of total dry matter of a crop which represents economic yield and has a value between 0 and 100% (Equation 2.1).

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

The plant growth regulator Moddus® increases the harvest index of Italian and perennial ryegrass seed crops by increasing the amount of harvested seed while maintaining the same amount of non-seed biomass at harvest (Rolston *et al.* 2007).

Harvest index is influenced directly by the plants reproductive capability and is known as floret site utilisation (FSU). FSU is the percentage of florets present at anthesis contributing to a viable seed yield. Based on the seed yield components the theoretical production (potential yield) can be calculated as;

Equation 2.2: Inflorescences m^2 x spikelet/ inflorescence x florets/ spikelet x FSU x average seed weight.

The actual seed yield in perennial ryegrass, is low and extremely variable. This is because seed loss can occur throughout the following processes: pollination, fertilisation, seed set and development, harvesting and seed cleaning (Table 2.2) (Elgersma 1985).

Table 2.2: Processes occurring after anthesis and associated losses that reduce floret site utilisation (Elgersma 1985).

Florets at anthesis	Losses
Pollination	Empty florets
Fertilization	Empty florets
Seed set	Empty florets
	Early abortion
Seed development	
- Early growth stage	Abortion
- Food reserve accumulation stage	Diseases
	Abortion
- Ripening stage	Diseases
	Shattering
Harvesting	Shattering
	Damage
Cleaning	Empty florets
	Light seeds
	Heavy seeds

Of the approximate 60% of florets produced that develop into seed prior to harvest, only 10-30% of these florets produce saleable seeds (Tretheway & Rolston 2009). The earliest work by Anslow (1964)

showed that the processes occurring in each individual seed head, from pollination onwards, frequently conflict with their effect on yield and seed quality.

Of major importance is the increased individual seed weight after seed set and their reduction in numbers owing to self-shedding. Seed weight depends on the position of the seed within the seed head with seed weight decreasing from basal to distal spikelets and from basal to distal seeds within the spikelets. Therefore, seeds located further from the source of assimilate (stem) appear to be lighter. In ryegrass, abortion may occur after fertilisation up to approximately 21 days after anthesis.

2.9 Plant growth regulators

The introduction of plant growth regulators (PGR) in the 1980's transformed the ryegrass seed industry. The earliest work revolved around the use of paclobutrazol (PP333) which showed an increase of 30-130% in seed yield. Due to the chemical nature of PP333, residuals were left in the soil and reduced the following crop yield. Thus, the adaptation of PP333 was not widespread. The introduction of trinexapac-ethyl (Moddus®) in 1999, a foliar absorbed PGR, revolutionised seed yields with up to a 50% increase in seed yield (Figure 2.5) being achieved (Rolston *et al.* 2004).
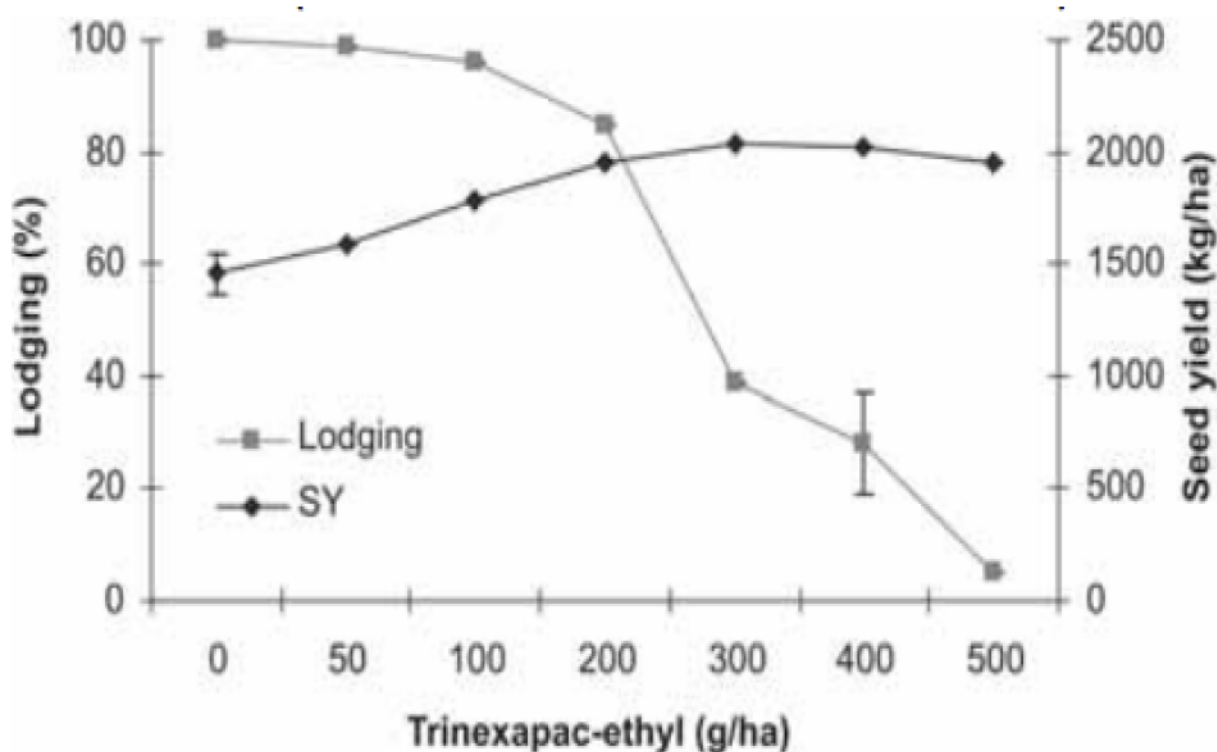
Figure 2.5: Seed yield and lodging response in dryland 'Nui' perennial ryegrass to rates of trinexpac-ethyl (Rolston *et al.* 2004).

These yield increases are similar to those achieved by Rolston *et al.* (2010) where a 44% yield increase was demonstrated. Plant growth regulators are applied with the objective of shortening and thickening the plants stem to reduce or delay lodging and to increase the number of ryegrass seeds per spikelet (Chynoweth 2010).
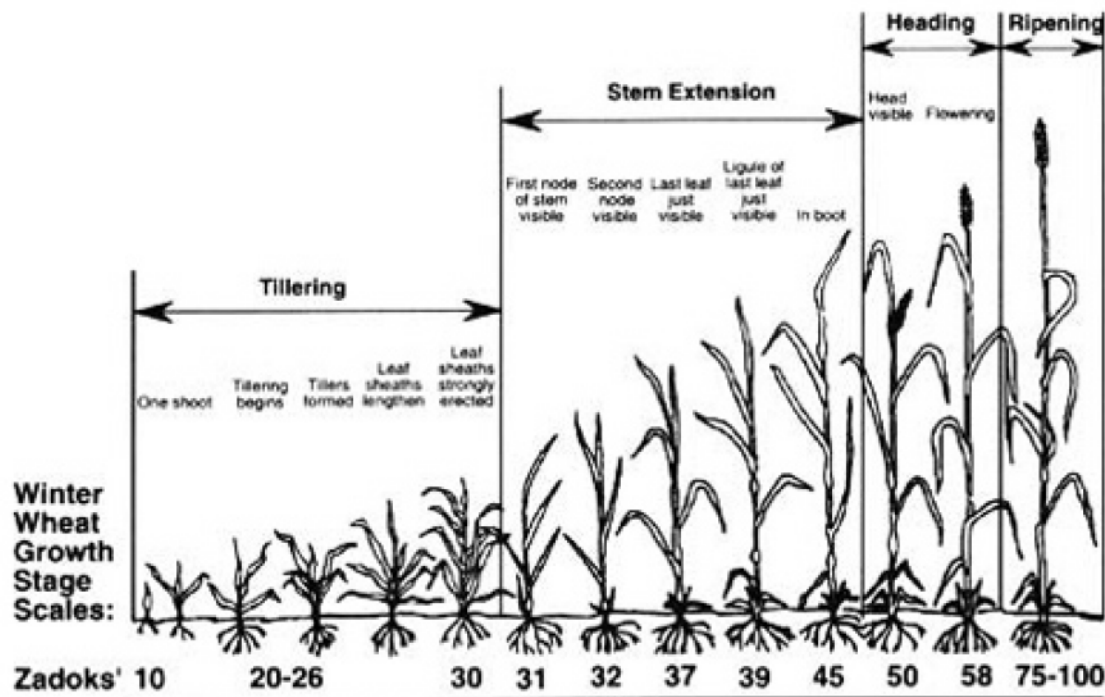
Figure 2.6: Growth stages of wheat in accordance to Zadoks growth stages (Zadoks *et al.* 1974).

In New Zealand, the current 'best practise' is to apply 1,200-1,600 ml/ha of Moddus® to perennial ryegrass seed crops and 800-1,600 ml/ha to Italian ryegrass seed crops (Chynoweth 2012; Rolston *et al.* 2012). The aim of Moddus® is to delay lodging until approximately two weeks prior to the expected harvest date and to increase harvested seed yield. Moddus's® mode of action is optimal when applied at Zadoks growth stage 32 (Figure 2.6) based on an assessment of the vegetative tillers (Zadoks *et al.* 1974).

Plant hormones are chemicals in small concentrations that promote and influence growth, development, and differentiation of plant cells and tissues. Gibberellins commonly referred to as gibberellic acid (GA) one of these plant hormones which plays a vital role in regulating seed germination, shoot growth, floral development and seed development. As GA affects the plants whole lifecycle, its biosynthesis occurs in multiple plant organs and cell sites at various times. GA is mobile and can act locally or distinct from sites of synthesis including young seedlings, shoot apices,

and developing seeds. GA is best known for its dramatic effect on promoting longitudinal growth (elongation) on internodes in grasses (Taiz & Zeiger 2010).

Applying Moddus® inhibits the GA pathway as an acylcyclohexanedione inhibitor of the 3-β hydroxylases. 3-β hydroxylases are one of the main enzymes responsible for the later stages of GA biosynthesis where GA is converted into a bioactive form. By inhibiting 3-β hydroxylases this decreases the bioactive GA production (Rademacher 2000). This causes the ryegrass stem to shorten and thicken as a result of limited stem elongation, leading to a reduction in total lodging or an offset in lodging (Rolston *et al.* 2007).

The chart shows Seed yield (kg/ha) on the y-axis versus Reproductive stem length (cm) on the x-axis, with a linear regression line: $y = -44.571x + 6253.1$, $R^2 = 0.92$.
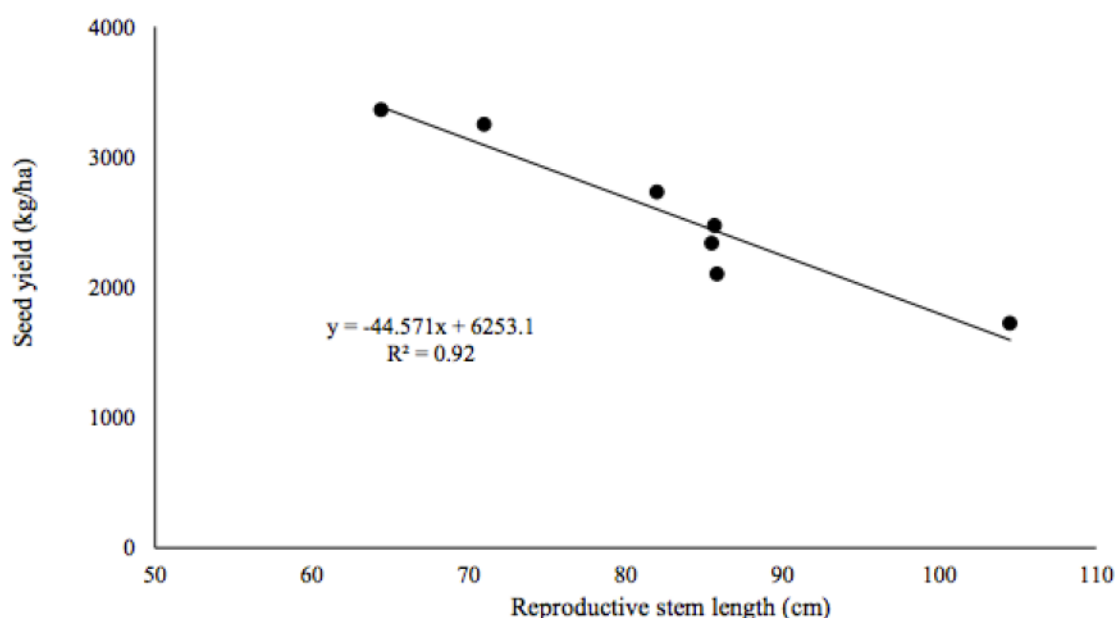
Figure 2.7: Influence of stem length on seed yield of perennial ryegrass cv. 'Bealey' following treatment with seven plant growth regulators when grown at Wakanui, Mid Canterbury in the 2013-14 season (Chynoweth *et al.* 2014).

Moddus® when applied at rates ranging from 0 to 3,200 ml/ha reduced stem length (P <0.001) from 105 cm in the untreated control to 85 cm. The shortest ryegrass stems produced the highest seed yield with an average 45 kg/cm of stem shortening (Figure 2.7) (Chynoweth *et al.* 2014). The Moddus® reduction in ryegrass stem length is consistent with data presented by Borm and van den Berg (2008) with ryegrass stems reduced from 103 cm in the untreated control to 88 cm in the highest treatment of 1,600 ml/ha.
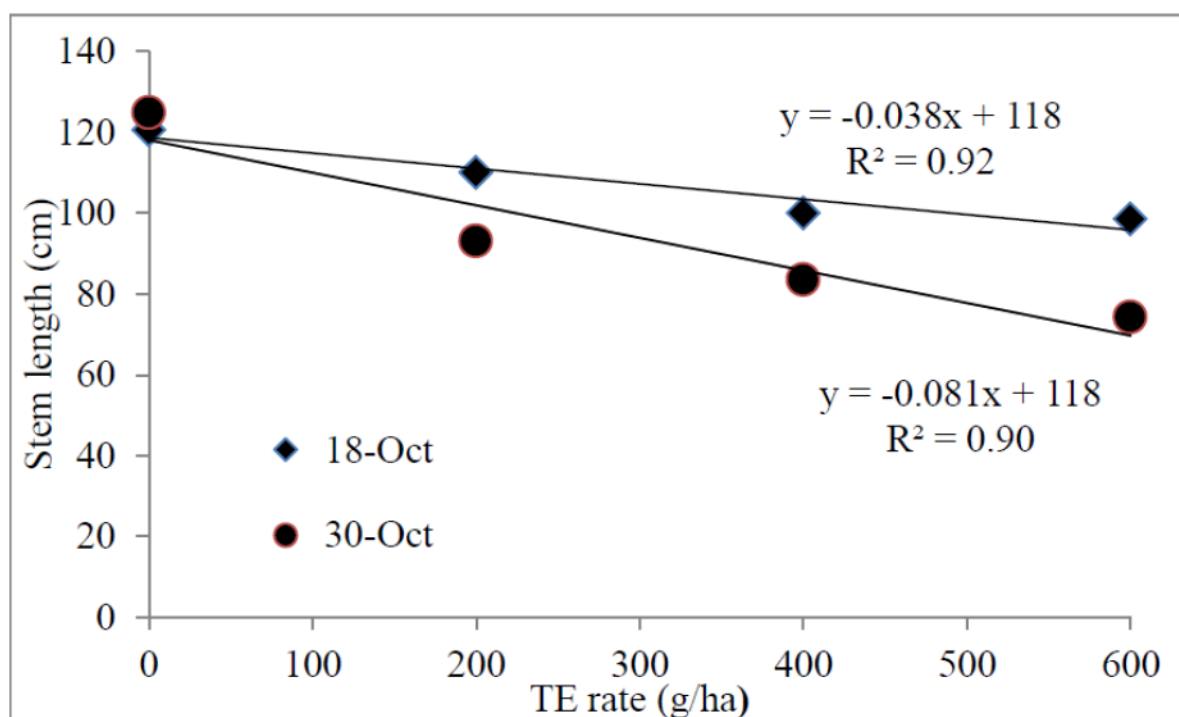
Figure 2.8: Change in reproductive stem length with increasing trinexapac-ethyl (TE) rates for two closing dates in cv. 'Crusader' at Milford (Rolston *et al.* 2012).

A similar trend has been demonstrated in Italian ryegrass seed crops when Moddus® rates ranged from 0 to 2400ml/ha (equivalent to 0-600 TE g/ha) in conjunction with closing date decrease stem length (P <0.001) (Figure 2.8). By delaying the closing day from the 18th of October to the 30th of October the Italian ryegrass response to the Moddus® application increased. The later closing date the amount of stem length decrease doubled, compared to the earlier closing with 8 cm per 100 g TE.

Moddus® usage and seed yield response in perennial ryegrass under New Zealand conditions are well documented (Rolston *et al.* 2004; Rolston *et al.* 2010). There are however, fewer reports on the response of Moddus ® in Italian ryegrass seed crops. When Moddus® was applied at rates ranging from 0 to 600 g/ha seed yield increased (P <0.001) by 29-64% over both sites with an optimum rate of 200-400 g/ha found (Rolston *et al.* 2012). Similar results were found by FAR (2013) with an Italian ryegrass seed yield increase from 27 to 58% when the application rate of Moddus® increased from 400 to 600 g/ha (Figure 2.9). The increase in Italian ryegrass seed yield was associated with shorter stems and delayed lodging, resulting in a greater reproductive efficiency assessed as saleable seeds per spikelet and harvest index.

Moddus® affects the number of seeds produced per spikelet through two mechanisms, either (i) increasing the floret numbers (Chastain *et al.* 2003) or (ii) increasing the percentage of florets that produce seed (Rolston *et al.* 2007), the latter being common in New Zealand.

However, limited work has been published which explains why or how a delay in lodging may increase seed yield in Italian and perennial ryegrass. Those that have, only describe lodging as hampering pollination and seed development (Hebblethwaite *et al.* 1978).
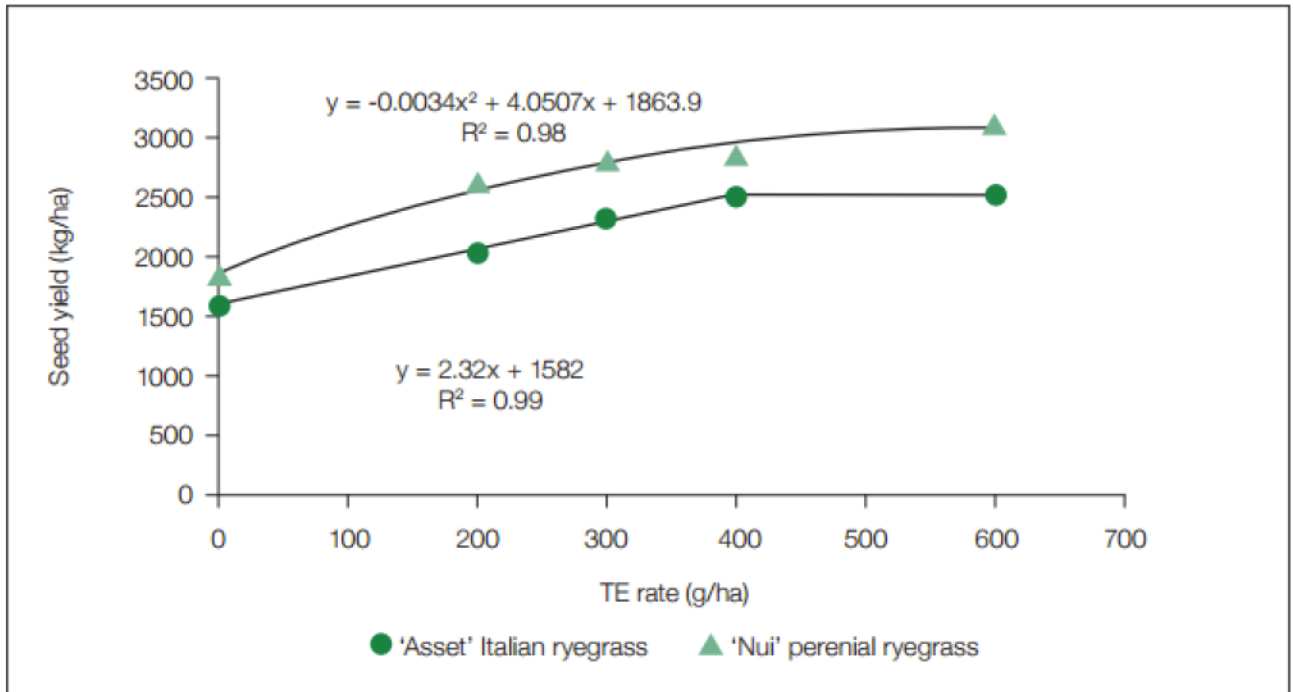
Figure 2.9: Trinexapac-ethyl (TE, Moddus) rate and seed yield response for Italian ryegrass cv. 'Asset' (●) and perennial ryegrass cv. 'Grasslands Nui' (▲) grown in Mid Canterbury during the 2012/13 growing season (FAR 2013).

2.10 Lodging

Lodging is the result of the structure of the culm (stem) being unable to support the increased weight of the developing spike and seed. As a consequence, the tiller and the spike 'lodges' or collapses to the ground under its own weight. Lodging is a universal problem in Italian and perennial ryegrass grown for seed. Lodging makes harvesting difficult with a large reduction in seed yield loss due to seed shedding and falling to the ground. This is especially the case under high nitrogen (N) fertilisation and soil moisture conditions with N and water promoting plant dry matter growth (kg DM/ha) and increasing seed yield (Rolston *et al.* 1994). This induces lodging as a result of the ryegrass plant unable to hold up its own weight and collapsing on itself (Chastain *et al.* 2014).

In Italian and perennial ryegrass seed crops, lodging can have adverse effects on seed yield contributing to a reduced photoassimilate supply to developing seeds, limited pollination and a lower seed set (Clemence & Hebblethwaite 1984). Depending on the timing of lodging, increased vegetative growth and lower utilisation of solar radiation can reduce overall seed yield (kg/ha) (Rolston *et al.* 2007). Pollination is reduced when lodging occurs pre-anthesis (flowering) with yield reductions up to 32% compared with a 15% reduction when lodging occurs two weeks after anthesis (Griffiths *et al.* 1980).

Griffiths (2000) investigated the effects of lodging on three cool season temperate grasses, including Italian and perennial ryegrass, through the use of poles and wires to support reproductive growth. Lodging reduced seed yield in Italian ryegrass in one season and perennial ryegrass in two seasons through reduced seed numbers. Lodging also reduced stem weight and inflorescence dry weight by an average of 25% compared with upright plants.

Table 2.3: Response of total stem dry mass and water soluble carbohydrates (WSC) to lodging in Italian ryegrass (*Lolium multiflorum* L.) and perennial ryegrass (*Lolium perenne* L.). Adapted from Griffiths (2000).

Species	Crop year	Condition	Seed number (per spike)	Seed mass (mg)	Seed yield (mg per fertile tiller)
Italian ryegrass	First	Upright	89	1.47	131
		Lodged	54*	1.68 *	91*
	Second	Upright	141	3.06	431
		Lodged	149	2.57*	383
Perennial ryegrass	Second	Upright	60	1.41	85
		Lodged	44*	1.50*	66*
	Third	Upright	84	3.57	300
		Lodged	52*	3.93*	204*

Significance (*) is at 0.05 level determined from ANOVA.

Water-soluble carbohydrates (WSC) were 30-63% less in stems and 37% less in the inflorescence when lodging occurred compared to upright plants. Lodging was associated with a depletion of stem

reserves during the grain filling period. When lodging occurred, seeds relied upon stored assimilates when the photoassimilate supply was limited.

A higher seed dry mass (mg) ($P <0.05$) occurred in perennial ryegrass in lodged plants (3.93 mg) compared to upright plants (3.57 mg). In contrast, seed number (per spike) was reduced under lodging (52) compared to upright plants (84). Perennial ryegrass suffered the greatest loss in seed yield from lodging (Table 2.3). Perennial ryegrass had the least total WSC reserves compared to the other temperate grass species studied. This supports the fact that seed filling is dependent on stored assimilates when the photoassimilate supply is limiting.

Compared to perennial ryegrass, Italian ryegrass, despite losing total dry mass (g) due to lodging, showed similar inflorescence WSC levels to the upright plant inflorescence. There was no difference between the rate of WSC accumulation between the upright and lodged Italian ryegrass inflorescences. This illustrates the degree that seed sinks can compete for available assimilates between the two ryegrass species. The lower yield depression in lodged Italian ryegrass indicates a higher compensation potential by partitioning more reserves assimilate, from leaves and stem to support seed growth and development. This potential doesn't seem to be present to the same degree in perennial ryegrass. These findings suggest that Italian ryegrass has the ability to compensate under reduced assimilate supply at a period where a high assimilate is required by the seed.

2.11 Conclusions

- While the amount of assimilates in the stem may not be limiting ryegrass seed yield, the distribution and remobilisation of available WSC is an important factor. This indicates processes within the seed head may be the driving factor in seed fill.

- GA is a plant hormone best known for its dramatic effect on promoting longitudinal growth (elongation) on internodes in grasses. Moddus® when applied at GS 32 can reduce stem length by inhibiting 3-β hydroxylases, one of the main enzymes responsible for the later stages of GA biosynthesis. This leads to ryegrass stem shortening and thicken.

- The shorter stems produced by the highest levels of Moddus® can produce the highest seed yields in Italian and perennial ryegrass. Shorter stems have a greater reproductive efficiency assessed by an increase saleable seeds per spikelet

- In comparison to other temperate grasses, perennial ryegrass suffers the greatest seed loss. Seed number per spike decreased under the lodged plant compared to upright plant.

- Italian ryegrass, despite losing total dry mass under lodging, showed similar WSC in their inflorescence compared to the upright plant. This provides no evidence that Italian ryegrass was severely affected by lodging.

- Lodged Italian ryegrass showed a lower yield depression compared to the perennial ryegrass. This indicates Italian ryegrass showed a higher compensation potential through the ability to partition more assimilates from leaves and the stem to support seed growth and development.

3. Material and methods

3.0 Experimental site

An experiment was carried out in Inverson Field (Block I3) adjacent to the Lincoln University Field Research Centre, Canterbury, New Zealand (43° 38′ S, 172° 28 E), 11 m above sea level. The soil is classified as a Wakanui silt loam with 180-350 mm of silt loam topsoil overlaying varying textual layers ranging from clay loam to sandy loams. Below 2 m in the soil profile is stony gravels and stones (Cox 1978).

The previous cropping history was one year wheat (*Triticum aestivum*), preceded by one year Caucasian clover *(Trifolium ambiguum)*. The paddock was sprayed off with Roundup (360 g/l glyphosate) and then mulched. The area was then subsequently rotary hoed and rolled with a 'Cambridge roller' followed by a plough. Prior to planting the area was top worked using a power harrow followed by a harrow and a 'Cambridge roller' to produce a fine but firm seed bed.

3.1 Experimental design

The experiment was a randomised block design with four replicates. The treatments were two crops and three rates of plant growth regulator Moddus® (a.i. 250 g/l Trinexapac ethyl) 0, 1,600 and 3,200 ml/ha, with 24 plots in total. The plots were sown with 'Samson' perennial ryegrass (*Lolium perenne L.*) on the 14th May 2015 and 'Progrow' Italian ryegrass (*Lolium multiflorum L.*) on the 16th September 2015. Both ryegrass cultivars were selected for their rapid and vigorous establishment and high rust tolerance.

'Samson' is a medium tillered diploid perennial ryegrass bred by AgResearch Grasslands. 'Samson' is a mid-season heading date cultivar reaching anthesis three days later than the standard 'Nui' perennial ryegrass which is defined as day zero.

'Progrow' is a medium tillered diploid Italian ryegrass. 'Progrow' is a late heading date cultivar reaching anthesis 13 days later that the standard Italian ryegrass 'Tama'.

3.2 Experimental area

Each individual plot was 2.1 m wide by 10 m long (21 m^2) and sown with a 'Flexiseeder' plot drill with 15.42 cm row spacing and at a target depth of 15 mm. Plots were 'Cambridge rolled' the same day as

planting. Perennial ryegrass was sown at 15 kg/ha for a target population of 600 plants $m^{2.}$. The non-allocated perennial ryegrass plots were then rotary hoed and 'Cambridge rolled' a week prior to planting to prepare a firm seedbed for the Italian ryegrass. The non-allocated plots were then sprayed with Roundup (360 g/l glyphosate) at 2 l/ha and followed with a roundup stick prior to emergence to kill any surviving perennial ryegrass plants. The Italian ryegrass was sown at 20 kg ha^{-1} to achieve a target population of 650 plants m^{-2}. Sowing rates differed between the two crops according to germination percentage (%), thousand seed weight (TSW) and expected field emergence. Sowing rates were calculated to obtain a target population of 650 plants m^{-2} for the Italian ryegrass (Equation 3.1).

Equation 3.1 Sowing rate (kg/ha) = $\dfrac{Target\ plant\ population\ x\ TSW\ x\ 100}{Germination(\%)x\ Emergence\ (\%)}$

3.3   Meteorological data

Data from the Broadfields meteorological station, approximately 2 km north of the experimental site (43°62'S, 172°47'E) were used to calculate mean monthly air temperature and total monthly rainfall data, (Figure 3.1). The long-term average monthly temperature and total monthly rainfall means recorded in the same location from 1975-2015 are also presented.

The long-term average rainfall for the site is 632 mm, however between January 2015 and December 2015, a cumulative total of 430 mm had fallen. With the exception of April, June, September and December exceeding the long-term mean by 33 mm, 19 mm, 16 mm and 4 mm respectively, all other months received 6 mm to 42 mm less rainfall than the long-term mean. A mean air temperature from January 2015 to December 2015 of 12.1°C slightly exceeded the long-term mean air annual air temperature of 11.7°C. Average monthly temperatures were within the normal range with the exception of January (18.2°C), April (13.6°C) and October (12.3°C).
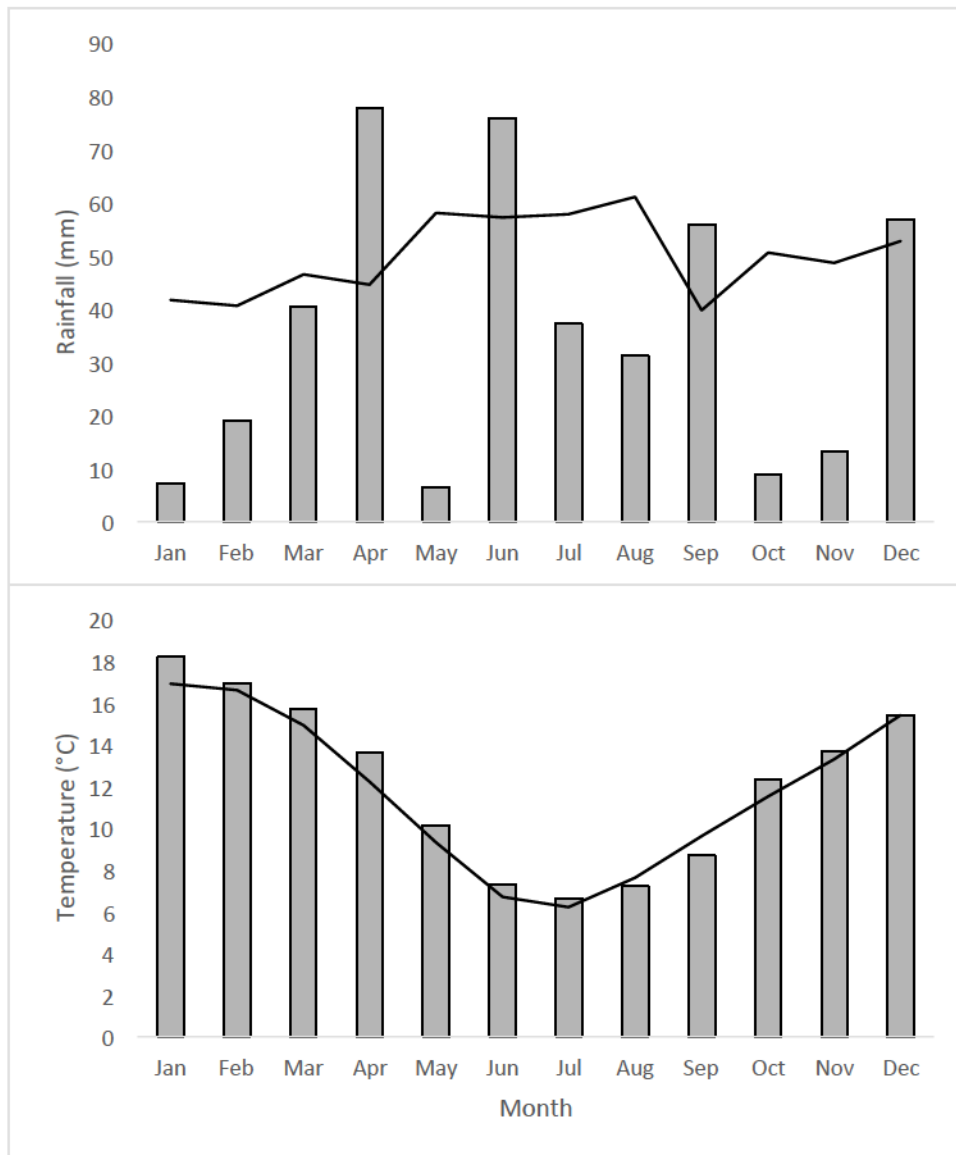
Figure 3.1: a) Total monthly rainfall and b) mean monthly air temperatures for 2015 growing season, with long-term mean from the Broadfield metrological weather site (43°62'S, 172°47'E).

3.4 In crop management

Broadleaf weed control was achieved for the perennial ryegrass seed crop by one spray of 4 l/ha Trimec® (a.i 600 g/L mecoprop, 150 g/L MCPA and 18.7 g/L dicamba) on the 30th October 2015. Further weed control in the ryegrass was achieved by hand grubbing in late spring/early summer. Broadleaf weed control was achieved for the Italian ryegrass seed crop by one spray of 3 l/ha Trimec® (a.i 600 g/L mecoprop, 150 g/L MCPA and 18.7 g/L dicamba) on the 19th November 2015. Moddus was applied at 0, 1,600 or 3,200 ml at Zadoks growth stage 32 (Zadoks *et al.* 1974). For both the perennial and Italian ryegrass on the 5th November and 2nd December 2015, respectively. An

average Zadoks growth stage of 32 was found by dissection and visually assessing the nodes. Spray application was made through a knapsack.

N was applied in a split of two applications based on nodal development with a target application of 120 kg N/ha. Soil mineral N was measured using a soil spear to a depth of 600 mm in 300 mm increments in September. The first N application of 160 kg/ha occurred on the 19th October as growth commenced in spring and was applied as Urea (46% N) using a tractor mounted boom spreader. The second application of 108 kg/ha occurred on 11th November and was applied as Urea (46%N) and applied the same as previously.

Disease control was achieved through two application of Proline® (a.i. 250 g/l prothioconazole) and Seguris Flexi® (a.i. 150 g/l Isopyrazam) between head emergence and end of flowering for preventative control of crown and stem rust in both the Italian and perennial ryegrass seed crops. All applications were made through a tractor-mounted boom sprayer delivering 200 l/ha using 04 flat fan nozzles at 300 kilopascals (Kpa) (Table 3.1).

Table 3.1: Fungicide product, application rate and application date for perennial and Italian ryegrass at Lincoln University, Canterbury in the 2015/16 growing season.

Plant species	Application dates, rate and product		
	20th Nov	10th Dec	23rd Dec
Perennial Ryegrass	4 L/ha Trimec®	400 ml/ha Proline® 600 ml/ha Sergus flexi®	800 ml/ha Proline® 600 ml/ha Sergus flexi®
Italian Ryegrass	3 L/ha Trimec®	400 ml/ha Proline® 600 ml/ha Sergus flexi®	400 ml/ha Proline® 600 ml/ha Sergus flexi®

Irrigation was applied as required to eliminate any chance of plant water stress. A water budget using accumulated evapotranspiration (ET) minus rainfall was used to determine water deficit. Irrigation was applied using a travelling rotating boom for a rate of 40-60 mm per application. Evapotranspiration was recorded from a National Institute for Atmosphere and Weather (NIWA) weather station located at Broadfields approximately 2 km north of the experimental site. Rainfall and irrigation applied were recorded at the experimental site using two rain gauges within the experiment (Table 3.2.).

Table 3.2: Irrigation timing, amount (mm) applied and when application occurred for Italian and Perennial ryegrass grown at Lincoln University, Canterbury in the 2015/16 growing season.

Irrigation date	Amount applied (mm)
19th October	45
27th October	60
20th November	50

3.5   Measurements

3.5.1   Anthesis

At and following anthesis (GS 60) a 350 mm row length across two drill rows sample was dug out of the ground every 3-4 days until maturity (GS 90) from each plot. At each sampling, samples were dug between 9 am to 10 am to minimise diurnal changes in WSC. After sampling, each sample from the individual plot was bagged and put into refrigeration for approximately a week at 4°C. After a week, each individual sample is cut at the root base, rolled and put into the oven to be dried (60°C, 72h). Then put into storage for approximately two months.

From each sample, a sub-sample of comparable tillers (20 tillers per sample) was taken for reproductive stem measurements (cm), which was further dissected into various vegetative and reproductive fractions (stem, rachis and seed). For the purpose of this experiment stem and rachis were combined and collectively known as 'stem components'.

3.5.2   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.

3.5.3   Extraction and analysis

Each sample was oven dried overnight at 62°C overnight and ground to pass through a 1.0 mm screen. The ground samples were then extracted for soluble sugars (%DM) and starch (%DM). Soluble sugars (%DM) were estimated by near infrared spectroscopy (NIR) and calibrated based on

an 80:20 ethanol: water extraction and colorimetric determination. Starch (%DM) was estimated by NIR and calibrated based on Enzymic Hydrolysis of starch and colorimetric determination of glucose.

3.5.4   Seed harvest

At 40% seed moisture content (determined by hand-collected samples by oven drying at 130°C for two hours) five sowing rows by 1 m in length (0.75m^2) were cut at ground level within each plot to obtain harvest seed yield and above ground dry matter. Within each 0.75 m^2 a subsample was removed for harvest component analysis (Refer to 5.6.3) and then returned to the bulk sample for seed yield analysis. Harvest dates were 6/1/16 for the perennial ryegrass and 10/2/16 for the Italian ryegrass. The cut samples were placed in bags and allowed to be dried naturally by hanging outdoors. After approximately 14 days bags were collected and stored indoors. Three months following harvest, samples were threshed using a Winterstieger stationary thresher with all seeds collected for further processing. The seed was cleaned using a screen cleaner for the removal of straw. All seed yields are reported at 12% seed moisture content.

3.5.5   Harvest components

Seed head number was determined by counting the number of reproductive heads present in a 150 g subsample from the total area (0.75m^2) harvested in each plot. The sample was then multiplied up to get heads m^2. The number of spikelet's/head was counted on 15 stems from each sample. Spikelets per m^2 were calculated by multipyling heads per m^2 by the average number of spikelets per head. Thousand seed weight was determined by counting 200 seeds from the final machine dressed sample and multiplied up by five.

3.5.6   Statistical analysis

All statistical analysis was performed using Genstat 16. All plot data were analysed using an analysis of variance (ANOVA) with means separated using the least significant difference test (LSD). For linear and non-linear regression, all individual plots were fitted in Genstat 16 to generate appropriate parameters. If all plots responded in the same way, treatments were combined across reps for curve fitting.

4.0 Results

For the first part of the result section the main focus is on Italian and perennial ryegrass yield components at harvest, harvest index and briefly on lodging at harvest. The second part of the result section is on the changes in dry weight and WSC contents of perennial and Italian ryegrass stem components and the changes in dry weight, WSC and starch content in the seeds from anthesis to harvest. The period from anthesis to harvest is stated as days after anthesis (DAA) in the following tables and figures.

4.1 Lodging

Table 4.1: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on crop lodging (%) at harvest for Italian and perennial ryegrass at Lincoln University, Canterbury in the 2015-16 growing season.

Species	Moddus® rate (ml/ha)			Species mean
	0	1,600	3,200	
Italian	45	22	17	28a
Perennial	97	50	15	54b
Moddus ® mean	71a	36b	16c	
Effect	P value	SEM	LSD 0.05	
Species	<0.001	1.01	3.2	
Moddus® rate	<0.001	1.24	3.9	
Species* Moddus®	<0.001	1.75	5.5	

Note: 50%=crop leaning 45°C, 100%=horizontal, Means within a column followed by the same letter are not significant (P >0.05).

The absolute level of crop lodging at harvest varied between Italian and perennial ryegrass (P <0.001) (Table 4.1). The application of Moddus® influenced the absolute level of crop lodging at harvest (P <0.001). In perennial ryegrass the application of Moddus® reduced crop lodging from 97% (effectively horizontal) for 0 ml/ha to 50% (leaning on a 45°C angle) for 1,600 ml/ha and 15% for 3,200 ml/ha ($LSD_{0.05}$=3.9%).

In the Italian ryegrass the application of Moddus® reduced crop lodging from 45% for 0 ml/ha to 22% for 1,600 ml/ha and 17% for 3,200 ml/ha ($LSD_{0.05}$=3.9%). Overall there was a species and Moddus® interaction (P <0.001) with absolute lodging greater in perennial ryegrass for 0 ml/ha and 1,600 ml/ha with similar lodging seen in 3,200 ml/ha in both ryegrass species.

4.2   Vegetative stem length
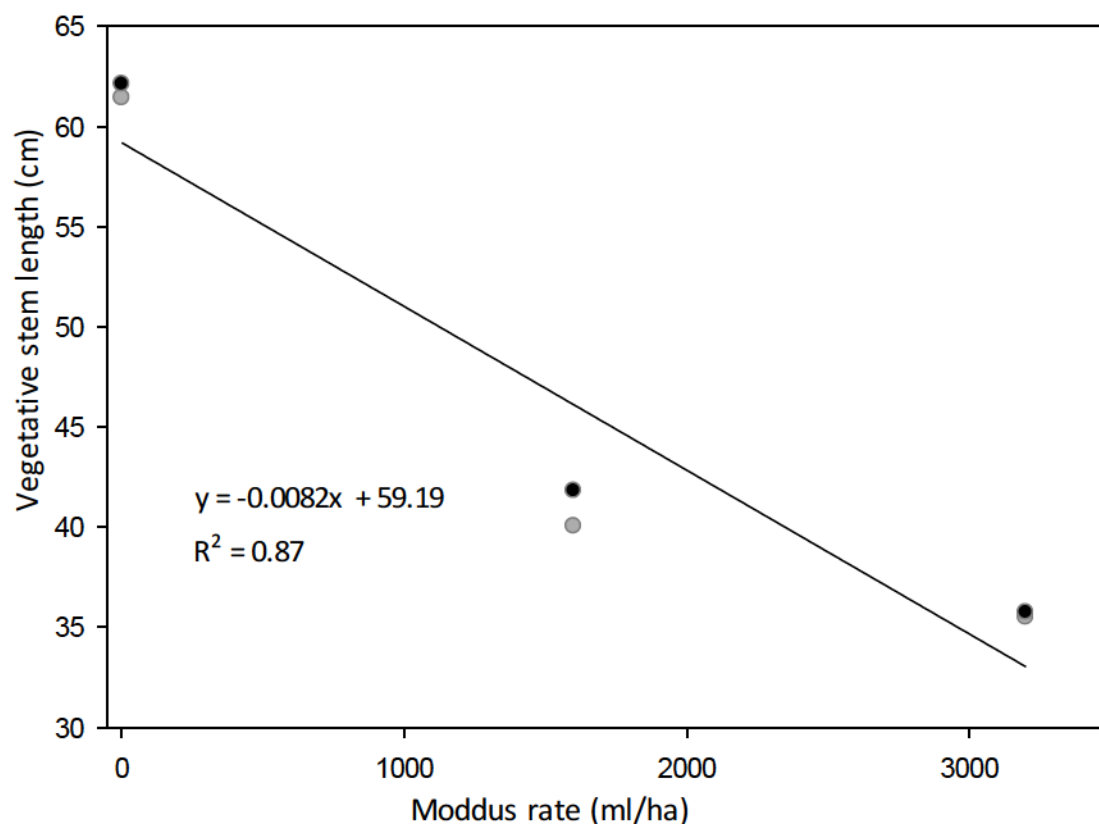
Figure 4.1: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) (0, 1,600, 3,200 ml/ha) on vegetative stem of Italian ryegrass (●) and perennial ryegrass (●) at Lincoln University, Canterbury in the 2015/16 growing season.

Vegetative stem length (cm) was reduced (P <0.001) in both perennial and Italian ryegrass by all Moddus® rates from 62 cm in the untreated control of 0 ml/ha to 35 cm with the highest Moddus® rate of 3,200 ml/ha (Figure 4.1). There was no difference on the effect of Moddus® between the Italian and perennial ryegrass vegetative stem lengths (cm).

4.3 Seed yield
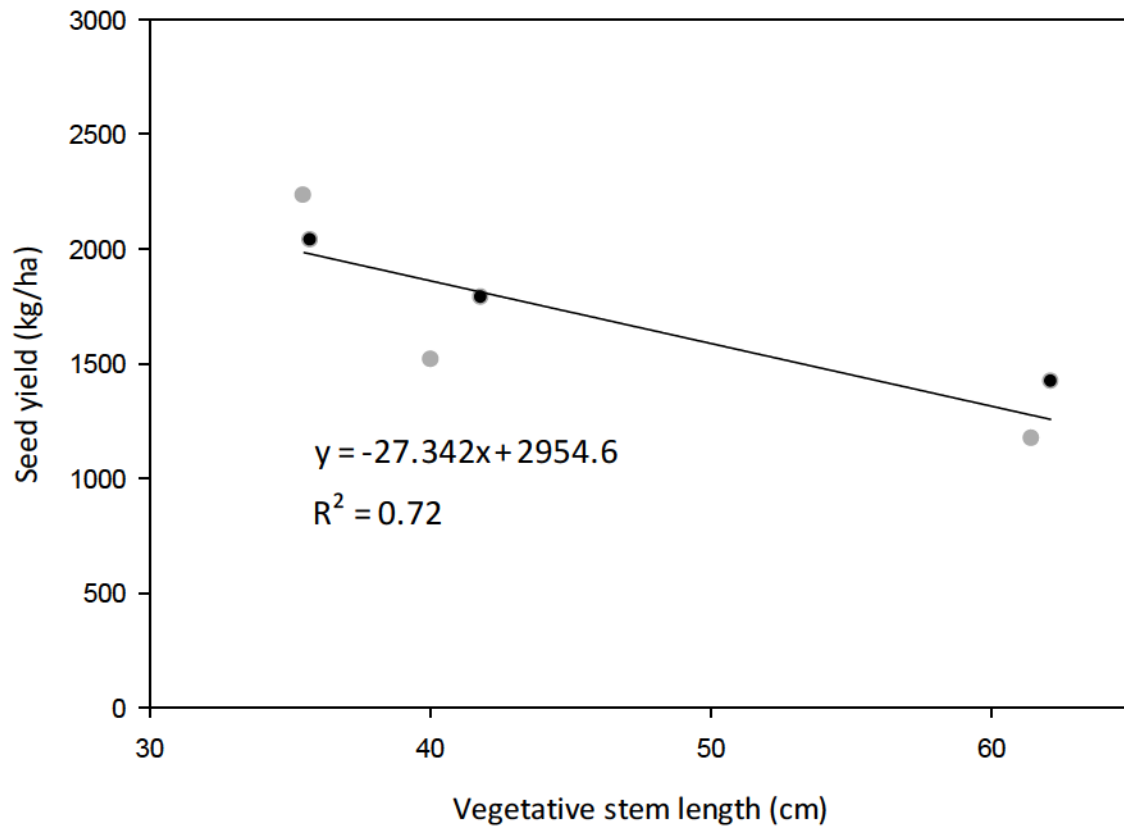
Figure 4.2: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) (0, 1,600, 3,200 ml/ha) on the vegetative stem length and seed yield of Italian (●) and perennial ryegrass (●) at Lincoln University, Canterbury in the 2015/16 growing season.

Seed yield (kg/ha) was affected ($P < 0.01$) by the Moddus® treatment but not by species and their interactions (Table 4.2). The application of Moddus® increased ($P < 0.01$) yield from 1650 kg/ha at 1,600 ml/ha to 2130 kg/ha at 3,200 ml/ha with similar yields between the untreated plots at 0 ml/ha and lowest Moddus® application of 1,600 ml/ha. The shortest stems produced the highest seed yields with an average 27 kg seed/cm of stem shortening (Figure 4.2).

Table 4.2: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on seed yield (kg/ha) of Italian and perennial ryegrass at Lincoln University, Canterbury in the 2015/16 growing season.

Species	Moddus® rate (ml/ha)			Species mean
	0	1,600	3,200	
Italian	1420	1790	2040	1748a
Perennial	1170	1520	2230	1640a
Moddus® mean	1300ab	1650bc	2130c	
Effect	P value	SEM	LSD $_{0.05}$	
Species	0.5	124	391	
Moddus®	0.01	152	479	
Species* Moddus®	0.5	215	677	

Note: Means within a column followed by the same letter are not significant (P >0.05).

4.4   Yield components

4.4.1   Heads/m^2, spikelets/head and spikelets/m^2

Seed heads per m^2 were affected by the species treatment (P <0.05), with perennial achieving 1790 heads per m^2 and Italian ryegrass 1430 heads per m^2 (LSD$_{0.05}$=196). Seed head per m^2 was not affected by the Moddus® treatment or their interactions (Table 4.3).

Spikelets per head were affected by the species treatment (P <0.001), with perennial achieving 19.2 spikelets per head and Italian 21.4 spikelets per head (LSD$_{0.05}$=1.041). Spikelets per head were affected by the Moddus® treatment (P <0.05) but not their interactions.

The number of spikelets per m^2 was not affected by any of the main treatments (Species, Moddus®) or their interactions.

Table 4.3: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on Italian and perennial ryegrass heads/m^2, spikelets/head and spikelets/m^2 at Lincoln University, Canterbury in the 2015-16 growing season.

Species	Yield component	Moddus® rate (ml/ha)			Species mean
		0	1,600	3,200	
Perennial	Heads/m^2	1,600	1750	2030	**1790a**
	Spikelets/head	19.57	18.9	19.23	**19.2a**
	Spikelets/m^2	31270	32230	38670	34060a
Italian	Heads/m^2	1330	1530	1430	**1430b**
	Spikelets/head	22.23	19.33	22.53	**21.4b**
	Spikelets/m^2	29560	29800	32260	30540a
Heads/m^2	P value	Spikelets/head	P value	Spikelets/m^2	P value
Species	0.002		0.001		0.143
Moddus®	0.095		0.016		0.177
Species*Moddus®	0.211		0.072		0.658

Note: Key figures in bold, means within a column followed by the same letter are not significant (P >0.05).

4.4.2 TSW

Thousand seed weight (TSW) was affected by the species treatment (P <0.001) but not by Moddus® or their interactions (Table 4.4). The large difference in TSW was driven by the species treatment, with Italian ryegrass having a larger TSW of 3.23 g compared with the perennial ryegrass 2.77 g (LSD$_{0.05}$ = 0.212).

Table 4.4: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on Italian and perennial ryegrass thousand seed weights (TSW) at Lincoln University, Canterbury in the 2015-16 growing season.

Species	Moddus® rate (ml/ha)			Species mean
	0	1,600	3,200	
Italian	3.24	3.15	3.30	3.23
Perennial	2.66	2.88	2.78	2.77
Moddus® mean	2.95	3.02	3.04	
Effect	P value	SEM	LSD 0.05	
Species	<.001	0.067	0.212	
Moddus® rate	0.714	0.082	0.259	
Species* Moddus ®	0.395	0.116	0.367	

Note: Means within a column followed by the same letter are not significant (P <0.05).

4.4.3 Seeds/spikelet

The number of seeds per spikelet was calculated from seed yield, TSW, seed head numbers and the spikelet/head data (Table 4.5). The number of seeds per spikelet was affected by Moddus® (P <0.05) as the main effect but not by species and their interactions. For Moddus®, 0 ml/ha produced 1.43 seeds/spikelet while the application of 1,600 ml/ha (1.80) and 3,200 ml/ha (2.03) both increased (P <0.05) the number of seeds/spikelet (LSD$_{0.05}$=0.48).

Table 4.5: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on Italian and perennial ryegrass seeds/spikelets at Lincoln University, Canterbury in the 2015-16 growing season.

Species	Moddus® rate (ml/ha)			Species mean
	0	1,600	3,200	
Italian	1.43	1.73	1.69	1.61
Perennial	1.43	1.87	2.38	1.89a
Moddus® mean	1.43ab	1.80bc	2.03c	
Effect	P value	SEM	LSD $_{0.05}$	
Species	0.141	0.124	0.389	
Moddus®	0.051	0.151	0.477	
Species* Moddus®	0.28	0.214	0.674	

Note: Means within a column followed by the same letter are not significant (P <0.05).

4.4.4 Seeds/m^2

Table 4.6: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on Italian and perennial ryegrass seeds/m^2 at Lincoln University, Canterbury in the 2015-16 growing season

Species	Moddus® rate (ml/ha)			Species mean
	0	1,600	3,200	
Italian	42.9	57.2	62	54a
Perennial	44.1	52.5	80.5	59a
Moddus® mean	43.5a	54.9b	71.2b	
Effect	P value	SEM	LSD 0.05	
Species	0.476	6.76	15.06	
Moddus®	0.023	8.28	18.44	
Species* Moddus®	0.382	11.7	26.08	

Note: Means within a column followed by the same letter are not significant (P <0.05).

The number of seeds per m^2 was affected by Moddus® (P <0.05) but not species and their interactions (Table 4.6). The application of Moddus® increased the number of seeds from 43.5 seeds per m^2 (0000) for 0 m/ha to 54.9 seeds (0000) per m^2 for 1,600 ml/ha and up to 71.2 seeds (0000) per m^2 for 3,200 ml/ha (LSD$_{0.05}$=18.44).

4.4.5   Harvest index

Table 4.7: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on Italian and perennial ryegrass harvest index (HI) at Lincoln University, Canterbury in the 2015-16 growing season.

Species	Moddus® rate (ml/ha)			Species mean
	0	1,600	3,200	
Italian	10	13	18	14
Perennial	8	10	16	11
Moddus® mean	9	12	17	
Effect	P value	SEM	LSD 0.05	
Species	0.031	0.0064	0.0203	
Moddus®	<.001	0.0079	0.0248	
Species* Moddus®	0.935	0.0111	0.0351	

Note: Means within a column followed by the same letter are not significant (P <0.05).

Harvest index (HI) assessed at final hand harvest, seed at 12% seed moisture content was influenced by both species (P <0.05) and Moddus® treatments (P <0.001) (Table **4.7**). Italian ryegrass had the greater HI with 14% compared to perennial ryegrass 11%. The application of Moddus® increased harvest index from 9% to 12% or 17% for 1,600 and 3,200ml/ha, respectively.

4.5   Stem component dry weight (mg)

Table 4.8: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on perennial ryegrass stem component dry weight (mg) from harvest to anthesis DAA at Lincoln University, Canterbury in the 2015-16 growing season.

Moddus® rate(ml/ha)	DAA						Moddus® mean
	0	9	16	23	30	37	
0	689	683	621	720	773	568	676a
1,600	676	678	778	733	714	674	709a
3,200	641	631	678	658	615	692	652a
DAA mean	668a	664a	692a	703a	701a	644a	
Effect	P value	SEM	LSD $_{0.05}$				
DAA	0.503	35.4	71.9				
Moddus®	0.093	25	50.9				
DAA*Moddus®	0.135	61.3	124.6				

Note: Means within a column followed by the same letter are not significant (P <0.05).

Perennial ryegrass stem components dry weight (mg) was not affected by the DAA or Moddus® and their interactions (Table 4.8).

Table 4.9: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on Italian ryegrass stem component dry weight (mg) from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015-16 growing season.

Moddus® rate (ml/ha)	DAA					Moddus® mean
	0	4	7	14	18	
0	618	657	592	506	555	586a
1,600	389	483	470	470	459	454b
3,200	402	368	368	342	355	367c
DAA mean	469a	503a	477a	440a	456a	
Effect	P value	SEM	LSD $_{0.05}$			
DAA	0.495	35.7	73.2			
Moddus®	<.001	27.7	56.7			
DAA*Moddus®	0.499	61.9	126.8			

Note: Means within a column followed by the same letter are not significant ($P < 0.05$).

Italian ryegrass stem components dry weight (mg) was affected by Moddus® ($P < 0.001$) but not by DAA or their interactions. The application of Moddus® decreased Italian ryegrass stem component dry weight (mg) from 586 mg in the untreated 0 ml/ha to 454 mg for 1,600 ml/ha application rate and 367 mg for 3,200 ml/ha application rate ($LSD_{0.05}=56.7$) (Table 4.9).

4.6   Seed dry weight (mg)

Figure 4.3: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) 0 ml/ha (●), 1,600 ml/ha (▲), and 3,200 ml/ha (■) on perennial ryegrass seed dry weight (mg) from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015/16 growing season.

Perennial ryegrass seed dry weight (mg) was affected by DAA (P <0.001) and Moddus® (P <0.05) but not their interactions (Figure 4.3). Perennial ryegrass seed dry weight (mg) was different from anthesis 0 DAA to 30 DAA, with maximum seed dry weight reached at harvest (37 DAA) with 177 mg (LSD$_{0.05}$=22.3). Similar perennial ryegrass seed dry weight (mg) was found between the untreated 0 ml/ha (133.5 mg) and 1,600 ml/ha application rate (127.9 mg) with a Moddus® influencing seed weight (mg) with 3,200 ml/ha application rate (155.8 mg) (LSD$_{0.05}$=15.77).

$$y = 178.24 + 7.66x + 0.052x^2$$
$$R^2 = 0.57$$

Figure 4.4: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) 0 ml/ha (●), 1,600 ml/ha (▲), and 3,200 ml/ha (■) on Italian ryegrass seed dry weight (mg) from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015/16 growing season.

Italian ryegrass seed dry weight (mg) was affected by DAA (P <0.05) but not by Moddus® and their interactions (Figure 4.4). Italian ryegrass seed dry weight (mg) was different from 4 DAA until harvest (18 DAA). Maximum seed dry weight was reached at 7 DAA with 200 mg ($LSD_{0.05}$=33.75).

4.7 Water-soluble carbohydrates (WSC) content of stem components

Figure 4.5: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) 0 ml/ha (●), 1,600 ml/ha (▲), and 3,200 ml/ha (■) on the water soluble carbohydrate (WSC) fraction of perennial ryegrass stem components from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015/16 growing season.

The water soluble carbohydrate (WSC) fraction of perennial ryegrass stem components (mg) was affected by Moddus® (P <0.001) but not by DAA and their interactions (Figure 4.5). The WSC fraction of the perennial ryegrass stem component (mg) was different between the untreated 0 ml/ha (327 mg) and the two Moddus® application rates of 1,600 ml/ha (408 mg) and 3,200 ml/ha (383 mg) ($LSD_{0.05}$=40.31).

Figure 4.6: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) 0 ml/ha (●), 1,600 ml/ha (▲), and 3,200 ml/ha (■) on the water soluble carbohydrate (WSC) fraction of the Italian ryegrass stem components from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015/16 growing season.

The water soluble carbohydrate fraction of the Italian ryegrass stem components (mg) was affected by Moddus® (P <0.01) but not by DAA and their interactions (Figure 4.6). The WSC fraction of the Italian ryegrass stem components (mg) decreased with the application of Moddus®. The untreated (0 ml/ha) WSC fraction of the Italian ryegrass stem component was (272 mg) compared to 1,600 ml/ha application rate, (209 mg) and the 3,200 ml/ha application rate (158 mg) ($LSD_{0.05}$=34.18).

4.8   Water-soluble carbohydrates (WSC) content of the seed

The equation shown in the figure:

$$y = 31.2 + 0.488x + 0.022x^2$$

$$R^2 = 0.52$$
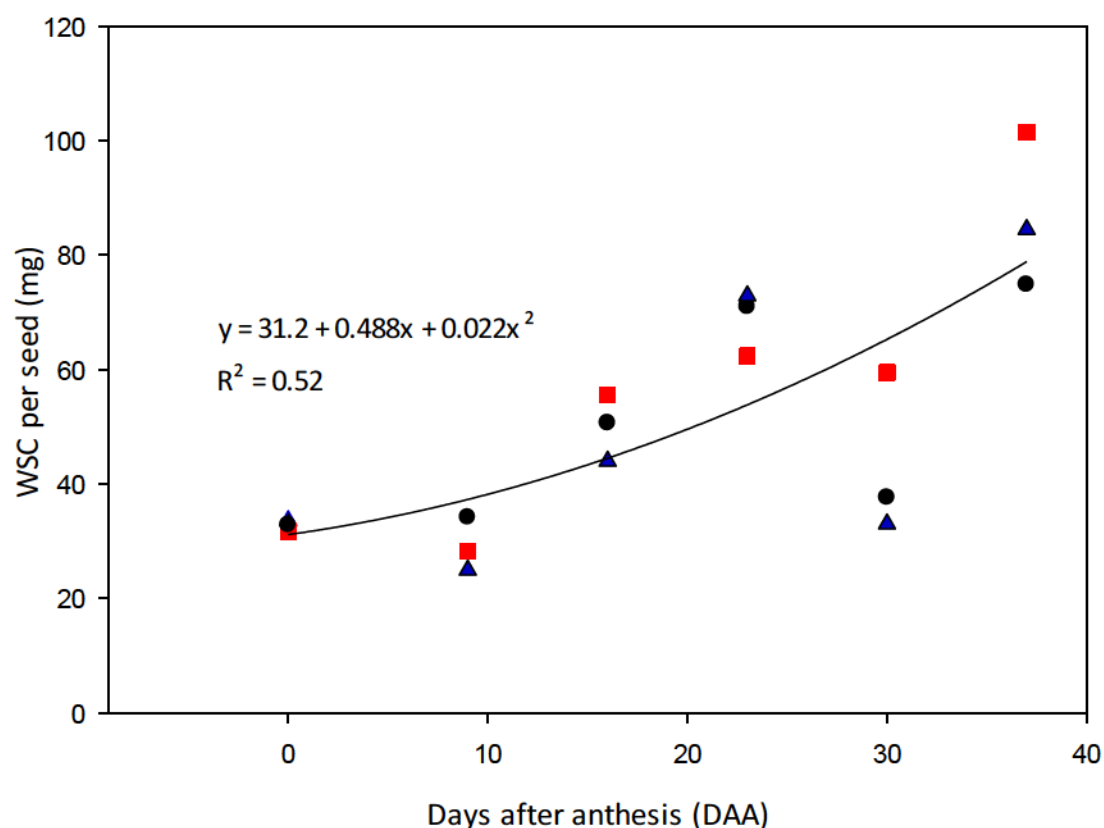
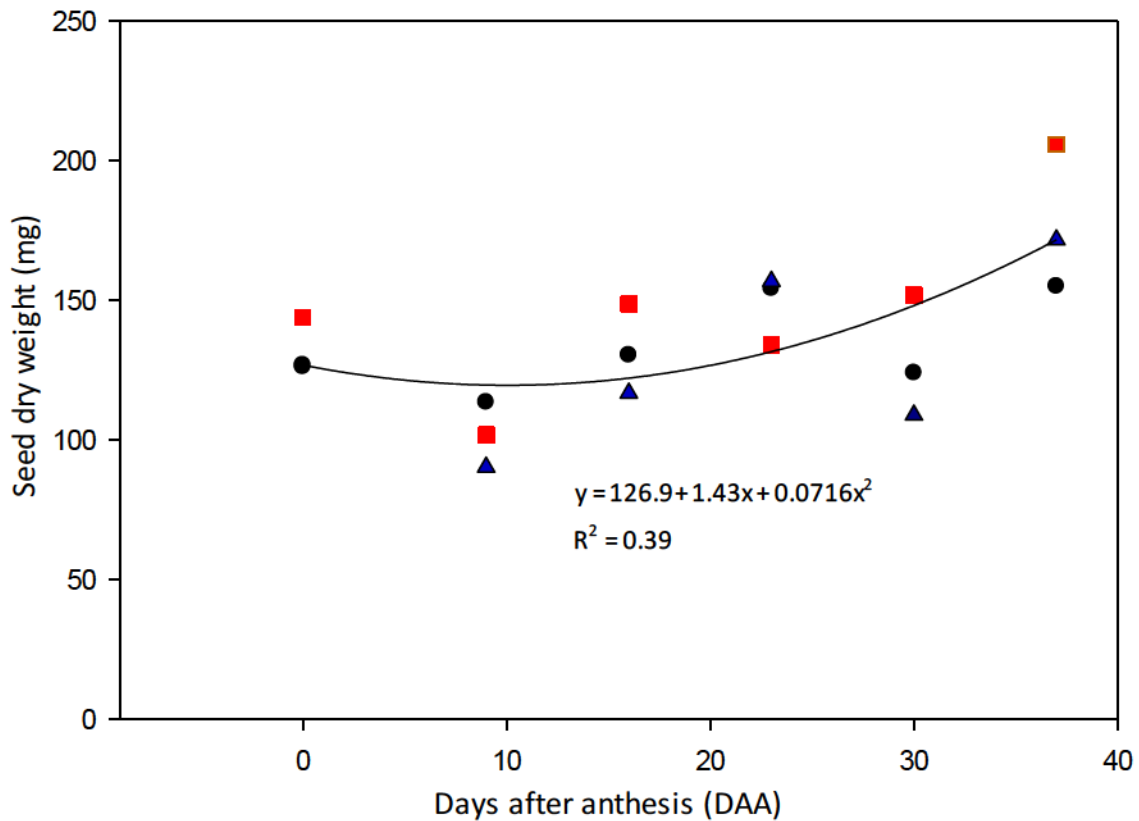**Figure 4.7:** Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) 0 ml/ha (●), 1,600 ml/ha (▲), and 3,200 ml/ha (■) on the water soluble carbohydrate (WSC) content of the perennial ryegrass seed from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015/16 growing season.

The water soluble carbohydrate (WSC) content of the perennial ryegrass seed (mg) was affected by DAA (P <0.001) and Moddus® (P <0.05) but not their interactions (Figure 4.7). The WSC content of the perennial ryegrass seed (mg) increased from 9 DAA until 23 DAA, with a maximum WSC content in the seed (mg) reached at 37 DAA (86.67 mg) ($LSD_{0.05}$=12.41). The WSC content of the perennial ryegrass seed (mg) was similar between the untreated 0 ml/ha (49.91 mg) and 1,600 ml/ha application rate (48.06) and increased in the 3,200 ml/ha application rate (59.63 mg), $LSD_{0.05}$=8.78.
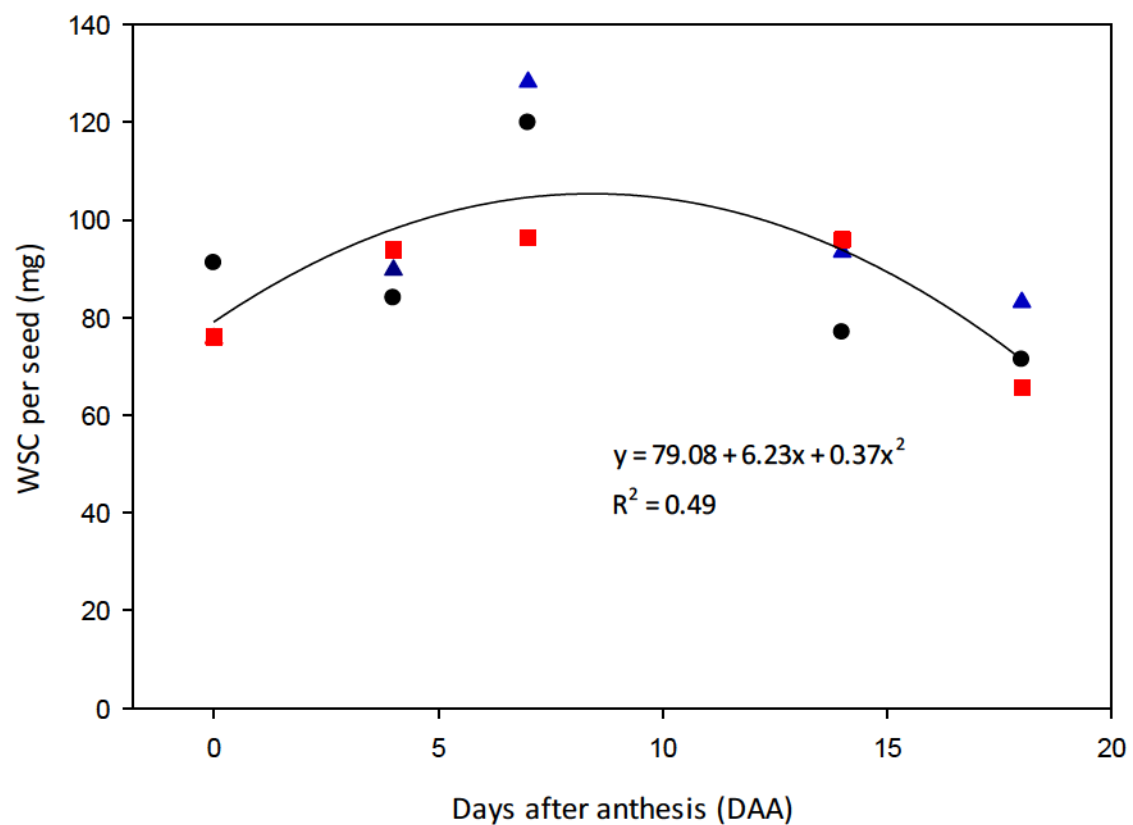
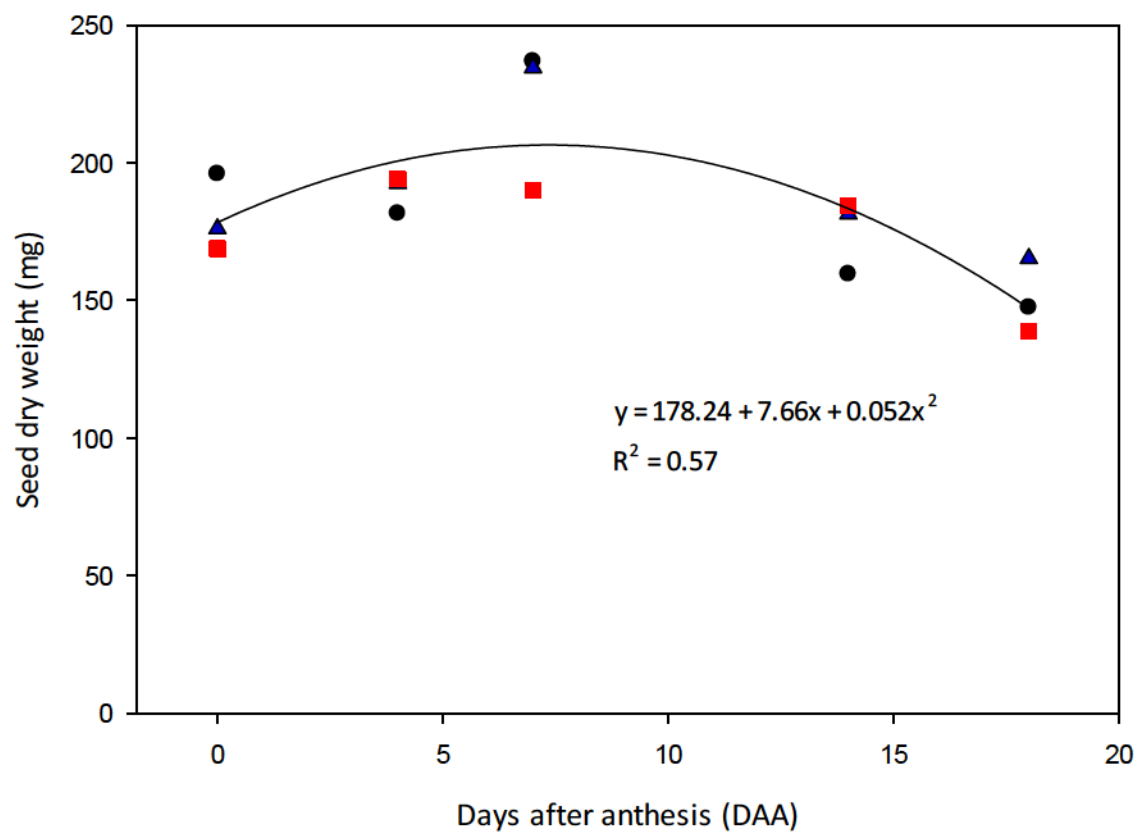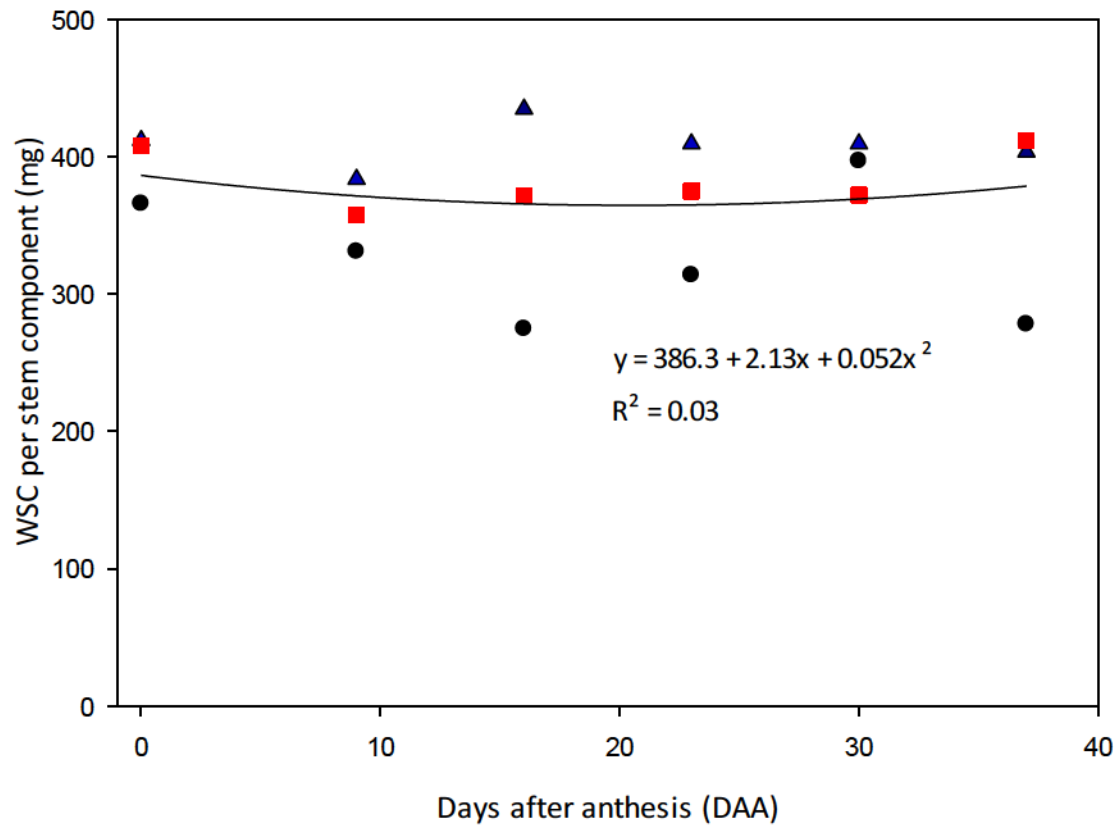**Figure 4.8:** Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) 0ml /ha (●), 1,600ml /ha (▲), and 3,200ml /ha (■) on the water soluble carbohydrate (WSC) content of the Italian ryegrass seed from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015/16 growing season.

The water soluble carbohydrate (WSC) content of Italian ryegrass seed (mg) was affected by DAA (P <0.05) but not by Moddus® and their interactions (Figure 4.8). The WSC content of the Italian ryegrass seed (mg) was greatest at 7 DAA at 115 mg and declined untill harvest ($LSD_{0.05}$=18.8).

4.9   Starch content of the seed
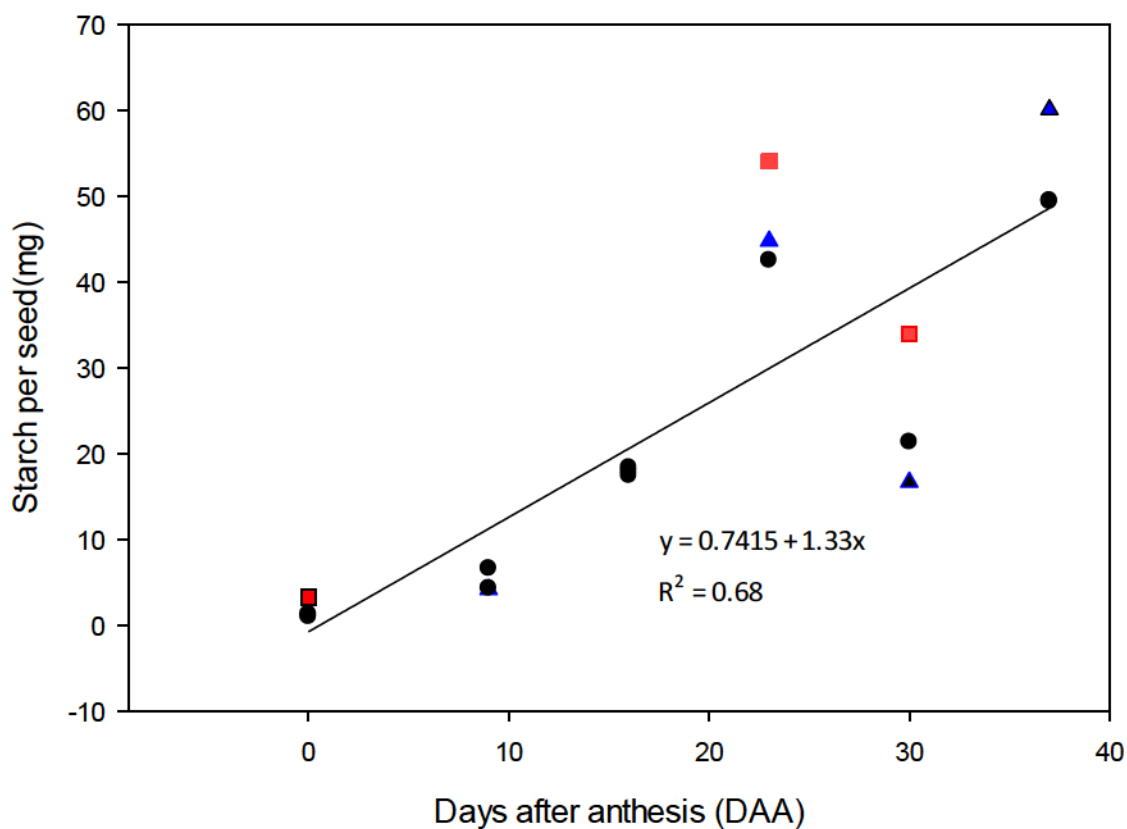
Figure 4.9: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) 0ml/ha (●), 1,600ml/ha (▲), and 3,200ml/ha (■) on the seed starch content of the perennial ryegrass seed from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015/16 growing season.

The starch content of perennial ryegrass seed (mg) was affected by DAA (P <0.001) but not by Moddus® and their interactions (Figure 4.9). The starch concentrations of perennial ryegrass seed (mg) increased from 9 DAA until 37 DAA, with maximum starch reached at 37 DAA (61.24 mg) (LSD$_{0.05}$=10.94).
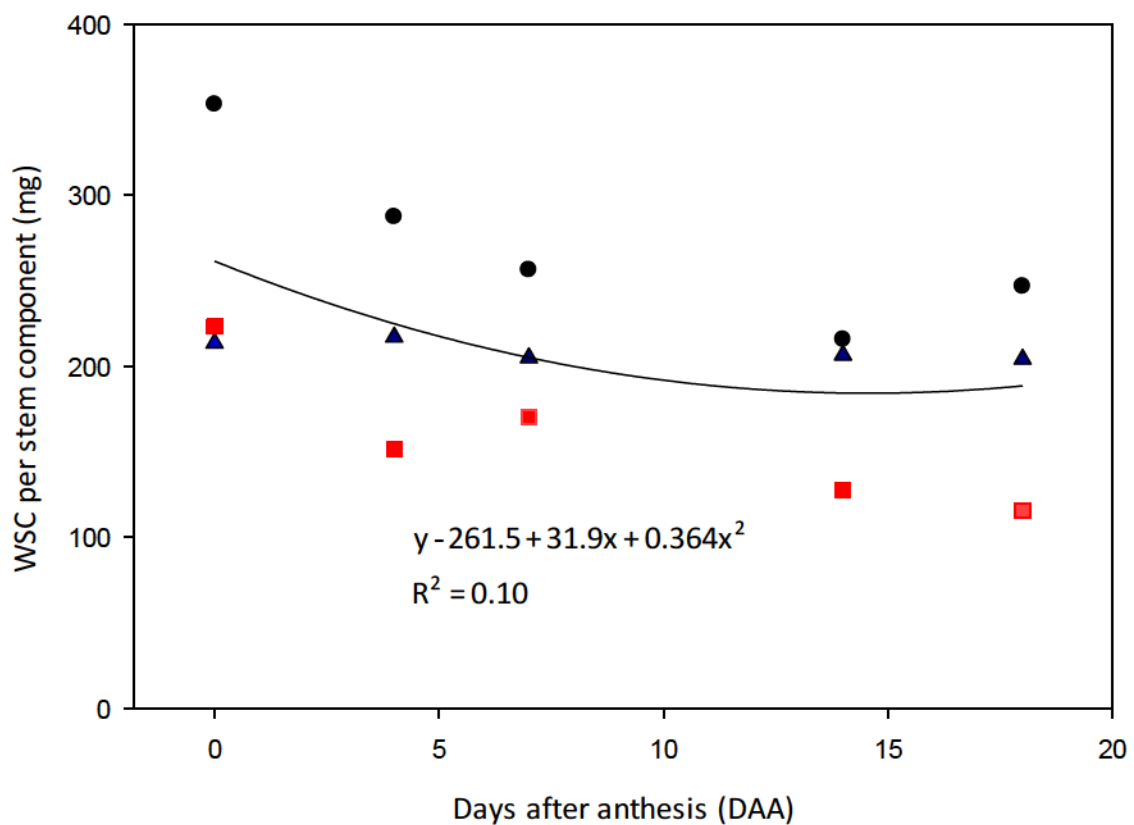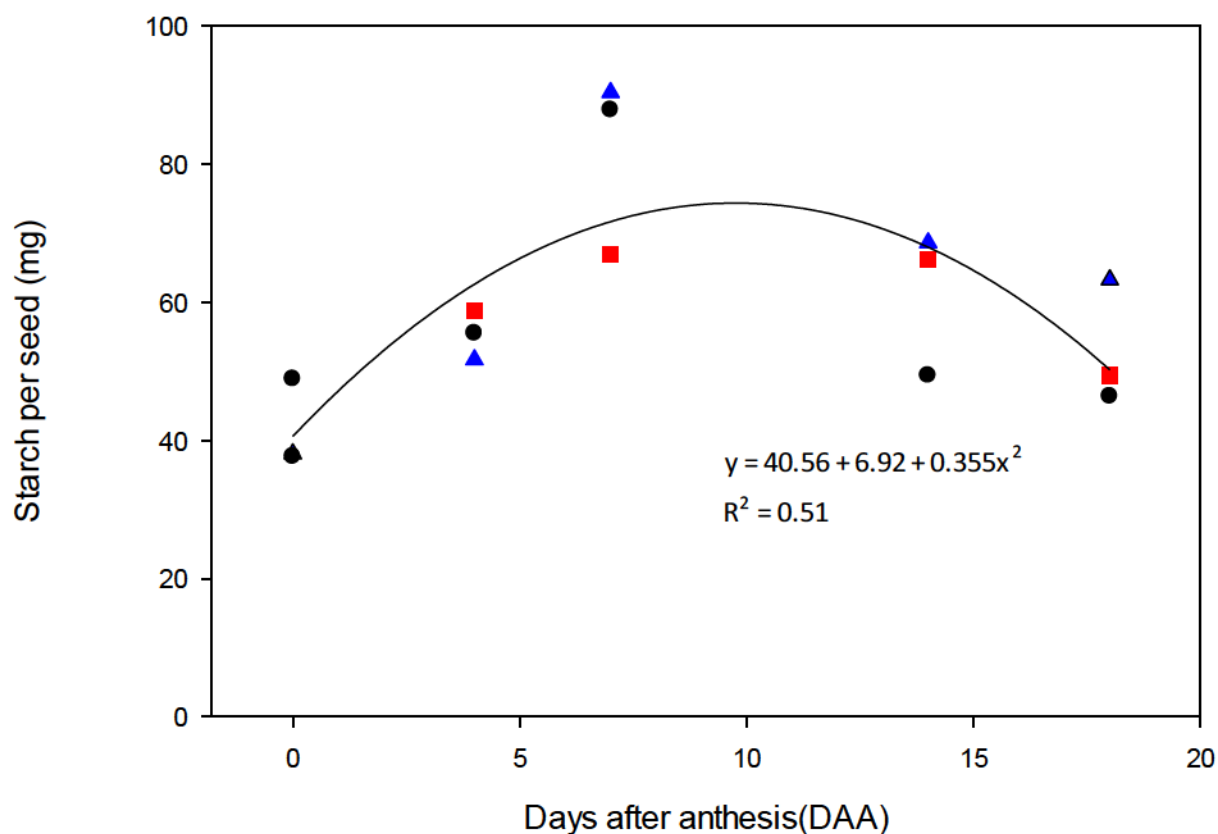
Figure 4.10: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) 0ml/ha (●), 1,600ml/ha (▲), and 3,200ml/ha (■) on the seed starch content of the Italian ryegrass seed from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015/16 growing season.

The starch content of Italian ryegrass seed (mg) was affected by DAA (P <0.001) but not by Moddus ® and their interactions (Figure 4.10). The starch content of Italian ryegrass seed (mg) increased from 4 DAA until 7 DAA (81.7 mg) and then declined untill harvest ($LSD_{0.05}$=15.15).

4.10   Starch content of stem components

Table 4.10: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on starch content of the perennial ryegrass stem components from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015-16 growing season.

| Moddus® rate | DAA | | | | | | Moddus® |
(ml/ha)	0	9	16	23	30	37	Mean
0	23.6	9.3	12.4	16.1	8.4	21.4	15.2
1,600	9.8	11.1	12.1	7.8	1.3	13	9.2
3,200	13.9	7.5	10.4	6.5	8	18.4	10.8
DAA Mean	15.8a	9.3a	11.6a	10.2a	5.9a	17.6a	
Effect	P value	SEM	LSD 0.05				
DAA	0.048	3.86	7.84				
Moddus®	0.09	2.73	5.54				
DAA*Moddus®	0.866	6.68	13.58				

Note: Means within a column followed by the same letter are not significant (P >0.05).

The starch content of the perennial ryegrass stem components (mg) was affected by DAA (P <0.05) but not by Moddus® or their interactions (Table 4.10). The starch content of the perennial ryegrass stem components were constant from 0 DAA through to 37 DAA ($LSD_{0.05}$=7.84).

Table 4.11: Effect of Moddus® rates (a.i. 250 g/l Trinexapac-ethyl) on starch content of the Italian ryegrass stem component from anthesis to harvest (DAA) at Lincoln University, Canterbury in the 2015-16 growing season.

| Moddus® rate | DAA | | | | | Moddus® |
(ml/ha)	0	4	7	14	18	mean
0	18.56	27.66	20.5	22.46	20.11	21.86ab
1,600	10.93	17.63	22.59	25.9	13.05	18.02b
3,200	14.88	11.75	13.65	15.54	6.53	12.47c
DAA mean	14.79a	19.01a	18.91a	21.3a	13.23b	
Effect	P value	SEM	LSD 0.05			
DAA	0.045	2.811	5.759			
Moddus®	<.001	2.178	4.461			
DAA*Moddus®	0.264	4.869	9.975			

Note: Means within a column followed by the same letter are not significant (P >0.05).

The starch content of Italian ryegrass stem components (mg) was affected by DAA (P <0.05) and Moddus® (P <0.001) but not their interactions (Table 4.11). The starch content of the Italian ryegrass stem component (mg) was constant from 0 DAA through till 14 DAA ($LSD_{0.05}$=5.76). There was no difference in the starch content of Italian ryegrass stem component (mg) in the 0 ml/ha (21.86 mg) and 1,600 ml/ha application rate (18.02 mg), However with Moddus® there was a difference with the 3,200 ml/ha application rate (12.47 mg) ($LSD_{0.05}$=4.46).

5.0 Discussion

5.1 Yield and yield components

Italian and perennial ryegrass vegetative stem lengths were similarly decreased by Moddus® (P <0.001), with no species difference (Figure 4.1 4.1). The application of Moddus® at the highest rate of 3,200 ml/ha reduced vegetative stem lengths by 26 cm compared to the untreated control. This reduction exhibits similar trends to the data presented by Chynoweth *et al.* (2014) where stems were reduced by 20 cm at 3,200 ml/ha. However, Chynoweth *et al.* (2014) reported these as reproductive stems including the seed head as part of the stem length measurement. Whereas the study undertaken, only the 'vegetative stem length' was measured excluding the seed head as part of the total measurement. There is limited literature on the influence of Moddus® on Italian ryegrass stem length at the higher rates of 3,200 ml/ha. Rolston *et al.* (2012) found an application of Moddus® at 1,600 ml/ha reduced reproductive stem length by up to 34 cm. As previously mentioned by Chynoweth *et al.* (2014),Rolston *et al.* (2012) measured 'reproductive stem length' and not vegetative stem length as measured in this study. The use of Moddus® as an inhibitor of the bio active GA reduced ryegrass stem length primarily by decreasing cell elongation, but also by lowering the rate of cell division within the stem (Rademacher 2000).

Shorter stems produced higher HI values (Table 4.7). Dry matter production for all treatments was the same, with potential sink size differences between Moddus® treatments. This suggests the sink in the highest Moddus® treatment had a greater capacity for assimilates during seed filling by the reduced stem competition (Tretheway & Rolston 2009). This implies a greater amount of assimilates is able to be partitioned from the stem and leaves (source) to the developing seed head (sink). This contributes to increased seed yield with a greater amount of assimilates transported to the seed reserves and stored as starch increasing the seed weight in the process.

Vegetative stem length was correlated (R^2=0.72) to seed yield (Figure 4.2). Seed yield was increased by Moddus® (P <0.01), with similar trends in both species. The shortest stems produced the highest seed yields, with an average increase of 27 kg of seed/cm of stem shortening. A similar trend was presented by Chynoweth *et al.* (2014) with the shortest reproductive stems producing the highest seed yield but with a greater average of 45 kg seed/cm of reproductive stem shortening. The differences in average yield increase were due to stem measurements taken and seed yield differences between studies. In Chynoweth *et al.* (2014) the highest yield of 3,360 kg/ha was achieved using a combination of plant growth regulators (Moddus, Paclobutrazol and Chlormequat chloride). In comparison to this study, the highest Moddus® application rate of 3,200 ml/ha achieved a relatively lower seed yield of 2,130 kg/ha.

Seed yield increased by 29% in both species when Moddus® rate increased from 1,600 ml/ha to 3,200 ml/ha, with similar yields between the 0 ml/ha and 1,600 ml/ha (Table 4.2). The number of seed heads/m^2 differed between the Italian (1,428 seed heads/m^2) and perennial ryegrass (1,793 seed heads/m^2) ($P < 0.05$). Italian ryegrass had a greater number of spikelets per head (21.37) ($P < 0.001$) than perennial ryegrass (19.23) (Table 4.3). However, a similar number of spikelets/m^2 was achieved in both species (despite a difference in both number of seed head/m^2 and spikelets per head. The differences in seed yield were associated with the crops ability to translocate assimilates to developing seeds. Therefore the differences in the harvested seed yield between the Moddus® treatments arose either from changes in individual seed weight or the number of seeds/spikelet increasing the number of seeds/m^2. There was no Moddus® effect on TSW in either species with 0 ml/ha (2.95 g), 1,600 ml/ha (3.02 g) and 3,200 ml/ha (3.04 g). However, TSW varied due to species differences with perennial ryegrass (2.77 g) and Italian ryegrass (3.23g) and the cultivars of ryegrasses chosen (Table 4.4). The Italian ryegrass 'Progrow' was a tetraploid with twice the number of chromosomes of the diploid perennial ryegrass 'Samson'. The increase in the number of seeds produced per spikelet was, therefore, responsible for the increase in seed yield in both species. In the untreated plots 1.4 seeds/spikelet were produced, increasing to 1.8 in the lower Moddus® rate plots (1,600 ml/ha) to 2.03 in the highest Moddus® rate (3,200 ml/ha) plots (Table 4.5). It has previously been shown that after a Moddus® application, the number of seeds per spikelet reaching a saleable weight increases in both ryegrass species in New Zealand and in the USA (Chastain *et al.* 2014; Rolston *et al.* 2010, 2012).

HI was increased by Moddus® ($P < 0.001$) and between the species ($P < 0.05$). Italian ryegrass (14%) had a greater HI than perennial ryegrass (11%) in this experiment, Moddus® increased the HI from 9 to 17 % due to increasing seed yield (Table 4.7). Moddus® when applied at a single application at the rates of 1,600-3,200 ml/ha showed no difference in dry matter production over all treatments in both species. This a result supported by Silberstein *et al.* (2003). The overall range of the HI (9-17%) shown in this work was low in compared to annual cereals crops (Hay 1995). Based on the genetic improvements seen in cereals, the HI of perennial ryegrass seed crops could be improved through plant breeding and crop management. However, a limitation of ryegrass seed crops is the number of tillers produced compared to annual cereals and thus the potential seed production difference. Ryegrass cultivars have generally been selected for tillering and dry matter production and thus produce more tillers than an annual cereal (Langer 1980). The order of tiller development has been shown to influence final grain weight produced per stem and an individual grain basis in ryegrass. Colvill and Marshall (1984) found older tillers produced the higher seed yields. The potential to improve the HI of ryegrass may be reduced with the number of second, third and fourth order tillers which produce seed heads.

5.2 Dry weight, water soluble carbohydrates (WSC) and starch content of plant components

5.2.1 Perennial ryegrass

The dry weight and WSC contents of perennial ryegrass stem components were consistent from anthesis to harvest (Table 4.8 4.8). Moddus® increased the WSC of individual stem components by 25% compared to the control. However, the WSC of stem component were similar in the Moddus® treatments. The consistent dry weight (644 to 703 mg) of the perennial ryegrass stem components from anthesis to harvest suggests that the seed requirements for assimilates were met by the amount of assimilates transported by the stem over this time period. However, the WSC concentration varied between the control (327 mg) and the Moddus® treatments, with a high amount of WSC remaining in the stems (383-409 mg) (Figure 4.5).

The shorter stems, produced by Moddus® produced higher HI but similar dry matter production between all treatments. In the Moddus® treatments the sink had a greater capacity or a greater proportion of WSC able to be translocated to the seed during grain filling. It would be expected that stem (source) competition was reduced through reduced stem length with the stem capacity to store assimilates decreasing as a result. However, it seems that the total WSC in the vegetative stem following anthesis is not limiting seed yield. A large amount of WSC is still available in the stem even though the seed yield increased relative to stem length (Figure 4.2).This species had the capacity to fill the available seed and accumulate WSC simultaneously, with little or no remobilisation of WSC from the stems, despite the decreasing stem (source) storage capacity. Similar results were recorded by Tretheway and Rolston (2009) with a large amount of 'storage' WSC found in the internodes of the stem at harvest. This suggests that, while the total amount of WSC is not limiting the seed yield, the distribution and remobilisation of available WSC to the seed is an important factor limiting seed yield.

Perennial ryegrass seed dry weight increased from anthesis to 30 DAA, with the maximum perennial seed dry weight of 177 mg being reached at harvest (Figure 4.3). The higher Moddus® application (3,200 ml/ha) increased individual seed weight by 22% compared to the 1,600 ml/ha and control 0 ml/ha application which showed similar seed dry weights. The seed WSC and starch concentrations showed similar trends (Figure 4.7; Figure 4.9). WSC concentrations increased from 9 DAA to 23 DAA, with a maximum being reached at harvest (87 mg). Starch concentrations increased from 9 DAA to 37 DAA, with the maximum being reached at harvest (61 mg).The starch content in this study increased at a similar rate to the WSC content from anthesis to harvest. The seed demand for assimilates are met with the seed weights increasing as the WSC content begins to increase (9 DAA) and converts to

starch. A similar pattern of WSC accumulation in perennial ryegrass has been seen by Griffiths (2000) where WSC post anthesis generally increased in all stem and inflorescence components in the upright plants.

A large WSC concentration (86.67 mg) remained in the seed at harvest with only 49% (86.67 mg) of the total seed dry weight (177 mg) converted to starch. This implies a seed (sink) limitation with the storage capacity of the grain saturated. The seed may be confined by its number of storage sites (number of grains and endosperm cells) or it's innate ability of the storage sites to accumulate assimilates. Physical resistance between the stem, and seed head or within the grain and endosperm could be limiting assimilate translocation within the seed (Hay & Porter 2006). Tretheway and Rolston (2009) reached a similar conclusion with seed head itself maybe an important factor driving seed grain filling.

5.2.2 Italian ryegrass

The dry weights of Italian ryegrass stem components remained constant (183 to 219 mg) from anthesis to harvest (Table 4.9) Moddus® decreased individual stem component dry weights by 37% at the highest rate of 3,200 ml/ha compared to the control. The WSC concentration declined gradually from anthesis to harvest (Figure 4.6). Moddus® decreased individual stem component WSC concentrations at the highest Moddus ® rate of 3,200ml/ha by 42% compared to the control. This suggests, the source and sinks relationship between the stem and developing seed head has been modified. A greater amount of WSC assimilates is able to be exported from the stem assimilate reserves and partitioned to the developing seed.

Italian ryegrass seed dry weight increased from 4 DAA to 7 DAA, with the maximum of 200 mg being reached at 7 DAA, it then declined until harvest (Figure 4.4). Seed WSC and starch concentrations showed similar trends to the dry weights (Figure 4.8; Figure 4.10). Seed WSC increased from 4 DAA to 7 DAA with the maximum of 114 mg being reached at 7 DAA. Seed starch followed a similar trend to the WSC, increasing from 4 DAA to 7 DAA with a maximum starch level of 82 mg at 7 DAA.

Italian ryegrass showed a similar trend to perennial ryegrass. A large WSC concentration (114 mg) remaining in the seed with only 43% (82 mg) of the total seed dry weight (200mg) converted to starch. This applying a sink limitation despite yielding similar to the perennial ryegrass. This illustrates the greater yield potential of Italian ryegrass as a tetraploid in theory has double the yield capabilities. In Italian ryegrass, maximum seed dry weight was achieved when maximum WSC and starch concentrations peaked at 7 DAA (Figure 4.4, Figure 4.8, and Figure 4.10). At the same time period (7 DAA) stem component WSC began to decline (Figure 4.6). This demonstrates that Italian

ryegrass stems may play a role in seed filling under conditions where assimilate supply is limiting. Under these conditions stem assimilates are remobilised to the seed reserves. Similar results were seen by Griffiths (2000), with similar WSC levels in the inflorescence, despite lodging, resulting in a lower seed depression between the lodged and upright control plants.

5.3   Lodging

The main effects of species and Moddus® were all implicated in the absolute lodging percentage at harvest (Table 4.1). Lodging progressed more quickly in perennial ryegrass than in Italian ryegrass. The species difference suggests a variation between their ability to resist lodging by their stem strength. Moddus® reduced overall lodging at the higher rate of 3,200 ml/ha, delaying the onset of lodging and its progression in the lower Moddus® rate. This result is similar to those presented by Silberstein *et al.* (2003), Borm and van den Berg (2008) and Rolston *et al.* (2012). Lodging has previously been reported to reduce seed yield through reduced assimilate supply to the developing seeds (Clemence & Hebblethwaite 1984) and to reduce pollination and lower seed set (Burbidge *et al.* 1978).

In this experiment, lodging reduced perennial ryegrass seed yield (Table 4.2) with less WSC in the seed in lodged control plants of 0 ml/ha (49.91 mg) compared to the highest Moddus® treatment of 3,200 ml/ha (59.63 mg). In contrast to perennial ryegrass, the Italian ryegrass showed similar seed WSC between the lodged control of 0 ml/ha (88.6 mg) and the two Moddus® treatments of 1,600 (94 mg) and 3,200 ml/ha (85.5 mg). A similar trend was seen by Griffiths (2000) on the effects of lodging on three cool season temperate grasses, including Italian and perennial ryegrass. Perennial ryegrass had the least total WSC reserves and suffered the greatest yield loss from lodging.

In comparison, Italian ryegrass WSC accumulation remained similar to that of the upright inflorescences. This illustrates the degree to which seed sinks can compete for available assimilates between the two ryegrass species. The relatively lower seed depression in the lodged Italian ryegrass indicates a higher compensation potential. Italian ryegrass illustrates the ability to partition more assimilate reserves from the leaves and stems to support seed growth and development. This potential does not appear to be present to the same degree in perennial ryegrass, with greater yield losses occurring under lodging in this species.

5.4   Conclusions

- Moddus® applied at a rate of 3,200 ml/ha reduced the stem lengths of Italian and perennial ryegrass by 26 cm.

- Seed yield in both Italian and perennial ryegrass was increased by 29% when the Moddus® rate was increased from 1,600 ml to 3,200 ml/ha through an increase in the number of seeds produced per spikelet.

- Perennial ryegrass had the capacity to fill the available seed (sink) and accumulate WSC simultaneously with little or no remobilisation of WSC from the stems.

- The seed heed is an important factor driving WSC partitioning to the seed and the conversion to starch. A greater understanding of transport mechanism is required to further increase yields

- In Italian ryegrass, maximum seed dry weight, WSC and starch concentrations peaked at the same period the stem components WSC declined (7 DAA). This demonstrates that Italian ryegrass stems may play a role in seed filling under conditions where assimilate supply is limiting.

- Moddus® reduced overall lodging at the highest rate of 3,200 ml/ha in both Italian and perennial ryegrass and delayed the onset of lodging at the lower rate of 1,600 ml/ha.

- Lodging was more prominent in perennial ryegrass with lower seed WSC concentrations in the 0 ml/ha control (49.91 mg) compared to the highest Moddus® treatment of 3,200 ml/ha (59.63 mg). In Italian ryegrass the control 0 ml/ha (88.6 mg) had similar seed WSC levels compared to the two Moddus® treatments 3,200 (85.5 mg and 94 mg respectively).

- Italian ryegrass had less of a seed yield loss up to lodging compared with perennial ryegrass. This species may have a greater yield compensation potential with the ability to divert more WSC reserves from the leaves and stem to support seed growth and development.

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