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Phenological development of perennial ryegrass in response to temperature and photoperiod

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A thesis

submitted in partial fulfilment

of the requirement for the Degree

of Doctor of Philosophy

at

Lincoln University

by

R.J. Chynoweth

Lincoln University

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Abstract of a thesis submitted in partial fulfilment of the requirement for the Degree of Doctor of Philosophy

Phenological development of perennial ryegrass in response to temperature and photoperiod.

By

R.J. Chynoweth

Following emergence, perennial ryegrass is a vegetative juvenile and goes through primary (PI) and secondary induction (SI) phases before becoming reproductive and exhibiting floral initiation (FI). Experimental and anecdotal evidence shows the duration of primary induction is influenced by temperature while photoperiod (Pp) may influence the duration of both primary and secondary induction. The range of flowering responses shown by perennial ryegrass genotypes may be characterized by different responses to Pp and temperature. The aim of this work is to understand and quantify the range of observed responses.

The influence of temperature and Pp on the development of perennial ryegrass was investigated using genotypes from different centres of origin grown under controlled environment and field conditions. Initial screening showed many accessions that originate from ~33 to 46° latitude became reproductive in constant 18°C, 20 h Pp, while few flowered in constant 18°C, 14 h Pp. These results demonstrate that flowering occurred in response to a long Pp, without prior PI.

Progress through PI was assessed at 4, 8, 12 and 18°C, in both 8 and 17 h Pp. Plants were subsequently transferred to non-primary inducing conditions, 18°C, 17 h Pp, that would fulfil the SI requirements, at two weekly intervals for up to 12 weeks. Flowering was determined as when 50% of plants produced seed heads following SI. FI was determined by tracking accumulated organ number (primordium plus leaves) relative to Haun Stage

(HS). The expected pattern was represented by three straight lines. The first period represents vegetative growth with a rate of ~2 primordium/HS. The second stage began at FI when the rate of primordium production increased. The final period was when primordium production ceased, which was represented by no further increase in the number of primordium/HS. FI and terminal spikelet (TS) were calculated as the inflection points for this relationship. Maximum temperatures for completing PI in an 8 h Pp and 17 h Pp (respectively) were 18°C and 18°C for 'Medea', 12°C and 4°C for 'Kleppe', 12°C and 8°C for 'Grasslands Nui' and 12°C and 12°C for 'Grasslands Impact'. Thus, a function was required to reduce the upper limit of effective temperatures as Pp increased. No treatment achieved FI prior to exposure to long photoperiods. Thus, optimum temperatures for progression through PI (V_{sat}) were defined from treatments exposed to short days (8h). V_{sat} was defined as when the number of leaves produced post transfer reduced to 4HS from the shortest treatment duration. For 'Medea', this was 18°C and V_{sat} occurred after 28 days exposure, 'Grasslands Impact' required 56 days at 12°C, while 'Grasslands Nui' and 'Kleppe' required 70 days between 4 and 12°C. V_{base}, the minimum exposure required at the optimum temperature for 50% of plants to flower, was 0 days for Medea, 23 days for 'Grasslands Impact' and 46 days for 'Kleppe' and 'Grasslands Nui'. After V_{sat} plants continued to produce primordium at the vegetative rate until they were transferred to 17 h Pp, when FI occurred. This confirmed exposure to a long Pp was an obligate requirement for flowering. Additionally, no plant achieved FI prior to obtaining HS4-5, which sets a base HS for photoperiod perception while the minimum number of main stem leaves was nine.

In the field, all four genotypes achieved primary induction from all autumn sowing dates prior to the shortest day. Subsequently FI was triggered by lengthening Pp at a genotype specific base Pp (Pp_{base}) of ~10.5 h for 'Medea', Grasslands Nui' and 'Grasslands Impact' and 12 h for 'Kleppe'. When FI occurred at Pp_{base}, Grasslands Nui', 'Grasslands Impact' and 'Kleppe', produced ~6.5 leaves compared with 'Medea' that produced 5.5 while all genotypes reduced towards four leaves when FI occurred at the saturating Pp (Pp_{sat}). Pp_{sat} were 14 h for 'Medea', 15.7 h for 'Kleppe', 15.6 h for 'Grasslands Nui' and 17 h for 'Grasslands Impact'. Therefore, combinations of Pp_{base} and the slope of Pp response separated the genotypes and described the time from FI to final leaf emergence. Concurrently the relationship between the number of leaves to emerge post FI multiplied

by the phyllochron determined the date of final leaf emergence. Since ~2HS remained to emerge at TS for all sowing dates, the mechanism reducing the duration from FI to final leaf emergence is a reduction in the HS duration from FI-TS as Pp increases.

Two modelling techniques were calibrated to incorporate vernalisation and Pp responses. These predicted competence to flower and final main stem leaf emergence to within 6 days for four genotypes sown on five dates between early autumn and late spring.

Keywords: ARCWHEAT, daylength, development, floral induction, phyllochron, primordium, *Sirius*, vernalisation.

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1 GENERAL INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is a grass (Poaceae/Gramineae) that originates from Europe with a natural range from the Atlantic Ocean to central Asia and North Africa (~30°N) to Northern Europe (~62°N). Perennial ryegrass was used as a pasture grass by early European pastoralists and as such was introduced by graziers to new settlements, especially those of temperate climates or those of higher altitudes in sub-tropical environments.

Perennial ryegrass is the most widely sown pasture grass used in New Zealand. It thrives in irrigated or summer moist dairy pastures where it is popular as a long term pasture species (Stewart *et al.* 2014). Perennial ryegrass is easy to manage for forage quality, generally produces at least 14 T DM/ha/year and when mixed with legumes provides a high quality pasture for animal performance. Alternative uses include as a turf grass for stadia, golf courses, recreation parks and domestic lawns in temperate climatic zones, particularly in Europe, North and South America and Australia. Perennial ryegrass is an important species in the rotation of arable farmers throughout New Zealand (NZ) where approx. 10-12,000 ha are grown annually for both domestic and export use (Chynoweth *et al.* 2015). The supply of seed is the delivery mechanism for improved forage and endophyte genetics to pasture and amenity markets.

The ability to predict flowering (anthesis) is important as this allows the implementation of agronomic management at predefined developmental stages. The time of flowering signals the switch from vegetative production to the start of seed filling in annual and perennial crops. This is preceded by a switch from producing vegetative organs (leaves) to producing reproductive organs (spikelet's and seed sites). The time of flowering can be predicted empirically from metadata in an environment using the mean of historical observations. Alternatively, it can be predicted mechanistically as a function of temperature accumulation and its modification by photoperiod (Pp) and vernalisation requirements. It is generally accepted that perennial ryegrass requires exposure to cool temperatures followed by long photoperiods for flowering to occur (Heide 1994).

Research aim and objectives

The aim of this thesis was to create a predictive model of the time to flowering of perennial ryegrass. To do this required the phenological development of perennial ryegrass to be quantified in relation to different temperatures and photoperiods. This was investigated through a series of controlled environment chamber and field experiments completed between 2016 and 2020. These had four objectives (Figure 1.1):

1. To identify germplasm with a range of responses to vernalisation, photoperiod and temperature treatments (Chapter 3).
2. To quantify the vernalisation and thermal time requirements for the phenophases up to final leaf emergence of four representative perennial ryegrass genotypes grown under long and short photoperiods (Chapter 4).
3. To quantify the timing of phenophases from emergence to flowering of four perennial ryegrass genotypes grown under field conditions with natural changes in photoperiod (Chapter 5).
4. To use the data collected in the previous experiments to determine what, if any, changes were required to adapt existing phenological models for wheat (*Triticum aestivum* L.), for them to be able to predict perennial ryegrass phenophases (Chapter 6).

Objective 1 was investigated through Experiments 1 - 4 which screened germplasm from a wide geographic origin ranging from Africa to Northern Europe. Lines with different induction mechanisms were selected from Objective 1 for use in later experiments to quantify vernalisation and photoperiod responses (Objectives 2 and 3). Objective 2 used controlled environment chambers to quantify phenological development using both (i) accumulated temperature (thermal time) modified by vernalisation and photoperiod and (ii) changes in the final number of leaves after exposure to different vernalisation and photoperiod treatments. In Objectives 2 and 3 the key assessments are the final number of main stem leaves and the number of primordium on the stem apex. Vernalisation is quantified from Experiments 5 - 12 to determine the influence of four temperatures on the rate of vernalisation under both short and long photoperiods. Analysis of the reduction in the number of main stem leaves and time of commitment to the final leaf allows calculation of cardinal temperatures, the duration required and the rate of vernalisation. Through analysis of the reduction in the final number of leaves and time of commitment of the final leaf, the rate of development along with vernalisation response and base photoperiod can be calculated. These are then be used in the development of

predictive equations. Counting of the number of primordium allows estimation of the inflection point in primordium production which is related to the time of commitment to reproductive development. With the addition of the number of leaves and the rate of primordium production, data can be used to estimate the final number of leaves. Objective 3 was met through Experiment 13 in which four genotypes were sown on five dates in the field to quantify the photoperiod response. Objective 4 proposes a framework to estimate final leaf emergence and flowering dates for perennial ryegrass. Thus, the resulting predictive model is the final step in achieving the aim. How the chapters fit together to meet the aim is outlined in Figure 1.1.

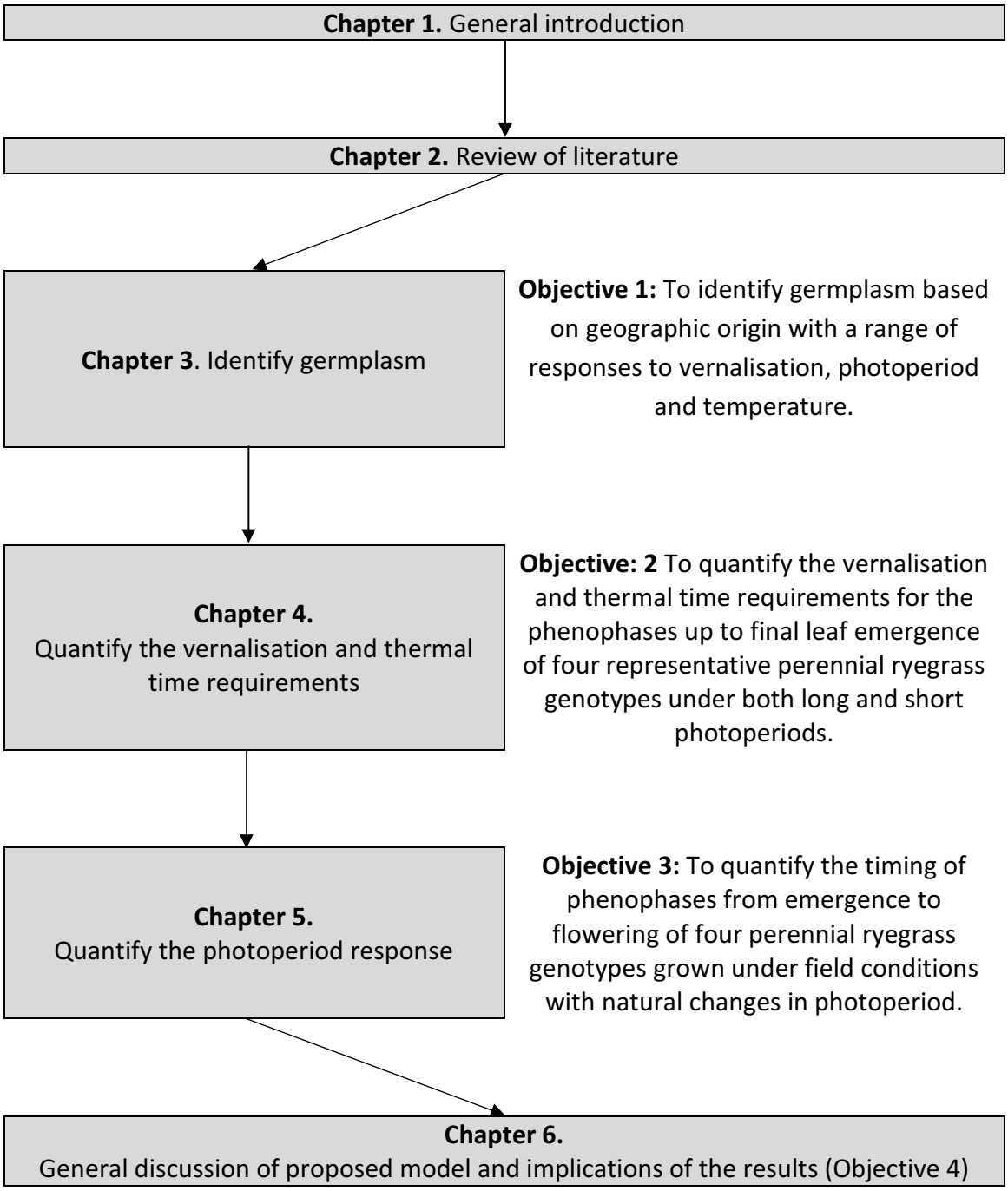


Figure 1.1. Flow diagram of this thesis structure.

2 REVIEW OF LITERATURE

2.1 Introduction

Reproductive development occurs when plants switch from solely producing vegetative organs to flower organs that are required for seed production. An understanding of when the switch to reproductive development occurs is important to match crop and cultivar performance with different climatic conditions. Also, in crop production, the time of stem elongation coincides with important agronomic management timings for nitrogen, and fungicide applications (Hay & Walker 1989) and plant growth regulators used to reduce stem internode length to encourage seed growth (Chynoweth *et al.* 2010; Chynoweth & Moot 2013). In pastures, stem elongation and ear emergence results in loss of pasture quality and a subsequent reduction in animal performance (Chapman *et al.* 2014). Thus, determining when the switch from vegetative to reproductive development occurs is important in seed production and grazing/mechanical defoliation to retain pasture quality. For clarity, throughout this thesis the term 'growth' refers to an increase in the size of plant organs and their weight due to photosynthesis. In contrast, 'development' refers to the progression of a plant through its life cycle or specifically the irreversible change from vegetative to reproductive primordium being initiated that results in seed production and physiological maturity (Hay & Walker 1989).

The progression of phenological phases is quantified by their relationship to abiotic (temperature and photoperiod) factors. These also influence crop growth which is driven by light interception from the canopy of leaves. During the vegetative phase, the priority for plants is leaf appearance which allows assimilate to be captured and used for canopy expansion and root growth. At some point in its life cycle, the plant will transition from vegetative development to reproductive development when the priority changes from leaf production to seed or grain growth.

This review of literature describes how grasses grow and develop to seed maturity and describes the environmental responses that plants undergo at different phases to progress from seed to a mature plant. It then identifies the requirements to quantify the

environmental response for wheat (*Triticum aestivum* L.) as a model plant used to inform the experimental investigation of perennial ryegrass.

2.2 How a grass plant grows

An individual ryegrass plant is made up of a collection of tillers where new shoots arise from buds within basal leaf sheaths and grow upwards, forming a bunch of tillers. Each tiller has three or four visible green leaves with the youngest leaf the most recent to emerge from the centre of the pseudostem, and the oldest closest to the plant base. Each leaf is made up of two parts, a leaf lamina (or leaf blade) and the leaf sheath. The leaf sheath wraps around the stem, or in vegetative plants forms the pseudostem. The pseudostem is a structure where individual leaf sheaths form concentric circles with the youngest leaf located in the middle. Within a tiller the basic repeating unit is called a phytomer. Each phytomer consists of a node, an internode, a leaf sheath and leaf blade and an axillary tiller and root bud (Moore & Moser 1995). In ryegrass, vegetative tillers have an active shoot apex which initiates the new phytomers with essentially no internode elongation. This leads to a stacking of nodes at the base of each tiller, at or below ground level where the apex remains protected from grazing animals. Each phytomer may produce a root if conditions are appropriate. Leaves are initiated on alternative sides of the apex and thus are on alternate sides of the pseudostem/stem when they emerge. After initiation of reproductive development, phytomer production ceases, the apex differentiates into an inflorescence (ear/head/spike) and the internode areas of phytomers with leaves yet to emerge begin to extend from oldest to youngest. When the internode areas are fully expanded, the tiller has reached its capacity for growth and once developing embryos have matured it will die. The initiation of phytomers is carried out at the apex in sequence and thus regulates the rate of their production. The inflorescence, or ear, of ryegrass contains between 5 and 40 spikelets which alternate sides on the central rachis. Each spikelet contains up to 12 seed sites.

2.3 Developmental scales

A number of crop development scales have been defined for cereals and these have been extended to a number of other crops under the "Biologische **B**undesanstalt, **B**undessortenamt und **CH**emische Industrie" scale or BBCH scale (Haun 1973; Zadoks *et*

al. 1974; Stauss 1994). Others have been specifically developed for visually assessing the transition from vegetative to reproductive development for perennial ryegrass (Sweet *et al.* 1991). Many agronomic inputs are based on the BBCH scale, an extension of the Zadoks scale of which many discrete stages can be estimated based on leaf emergence as these are coordinated events (Kirby 1990). For example, in wheat, fungicides are commonly applied to protect the 3rd to final leaf which is fully emerged (ligule visible) at Zadoks Growth Stage 32 (two nodes detectable) when the tip of final leaf 2 is emerging. Alternatively, the Haun stage (HS) provides a method to track the number of main stem leaves produced including the proportion of the emerging leaf and thus provides a more continuous scale (Haun 1973).

2.4 Phenophases and Phenology

The life cycle of perennial ryegrass, can be broken into seven phenophases that can be used to track developmental progression throughout the lifecycle. Throughout this thesis these will be referred to as phases 1 – 7 where predominately progress from one phase to the next is driven by thermal time accumulation modified by cool temperature and photoperiod requirements. As a starting hypothesis, this thesis assumes perennial ryegrass follows the same basic progression as wheat. Where necessary adjustments have been made to account for probable obligate requirements for primary and secondary induction (Heide 1994) that confer perenniality for ryegrass (Figure 2.1).

Table 2.1. Stage and phase names used throughout this thesis to describe progress from seed germination until maturity.

Stage Name	Phase Name		Phase length adjusted by:
Germination			
	Phase 1.	Emergence	Sowing depth
Emergence			
	Phase 2.	Primary induction	Cool temperatures and short photoperiod
Vernalisation complete			
	Phase 3.	Secondary induction	Long photoperiod
Floral initiation			
	Phase 4.	Ear development	Long photoperiod
Terminal spikelet			
	Phase 5.	Stem extension	-
Final leaf emergence			
	Phase 6.	Ear emergence	-
Flowering			
	Phase 7.	Seed filling	-
End Grain Fill			

2.4.1 A brief description of wheat phenology

Wheat genotypes can typically be described as either photoperiod (Pp) sensitive, or vernalisation-insensitive, 'spring' wheats and those that are vernalisation-sensitive or 'winter' types. Vernalisation-insensitive types produce a minimum number of main stem leaves when grown in long Pp, usually 6-7 leaves (Levy & Peterson 1972) requiring one true leave to perceive the Pp (Cooper 1956a). A wheat seed has 2-3 leaf primordium present with the 5th or 6th primordium being formed as the first leaf emerges (Hay & Kirby 1991), thus commitment to flowering is rapid under long Pp. In contrast, winter wheats produce leaf primordium during vernalisation and express 'juvenile' type characteristics

such that the final number of leaves is reduced as the duration of vernalisation is extended, until saturation occurs after which the leaf number remains constant (Cooper 1956a; Brooking 1996).

Development on the main stem, the first stem to emerge, follows the phenophases described in Table 2.1. Primordia accumulate on the stem apex and differentiate into final organs either before or during the production of a true stem with a variable number of leaves culminating in the production of an ear. This is followed by flowering, grain filling and physiological maturity (Kirby 1990). The appearance of leaves on the main stem is described by a linear function of temperature (Kirby 1995) from which the inverse is the phyllochron, the interval between two successive leaves. Primordium production is coordinated closely with leaf production such that leaf appearance can be used to estimate the number of primordium present on the stem apex (Kirby 1990; Brooking & Jamieson 2002). This becomes more accurate when leaf appearance is converted to the decimal Haun stage (Haun 1973). The time interval between the initiation of primordium is the plastochron (Wilhelm & McMaster 1995). However, the rate of primordium production is not constant and has two distinct phases, a slower 'vegetative' phase where leaf production has priority and a faster, 'reproductive' phase as spikelet primordium are developed (Brooking 1996). Spikelet and leaf primordium production ceases at terminal spikelet. This pattern creates two important inflection points:

1. at floral initiation (FI), and
2. at terminal spikelet (TS).

The formation of double ridges (DR) (Figure 2.1), described for ryegrass by Sweet *et al.* (1991), provides the first visible confirmation of reproductive development and occurs sometime after FI. The timing of FI occurs when the vernalisation requirement is saturated. However, this does not mean that all primordium produced at DR will become spikelets as a number of the lower primordium may become either leaves or spikelets (Griffiths *et al.* 1985; Brooking & Jamieson 2002). In ryegrass, DR begins in the centre of the apex with spikelet formation moving up and down the ear (Sweet *et al.* 1991). All primordium above the first DR become spikelets, culminating in the TS, similar to wheat

as described by Bonnett (1936). Below the first visible DR, and above those primordium that have committed to becoming leaves, are a number of primordia that may become either leaves or spikelets (Figure 2.1) (Griffiths *et al.* 1985; Brooking & Jamieson 2002). The HS at which TS formation occurs is closely related to the final number of leaves produced. Thus the fate of the final primordium is determined at the time of TS (Jamieson *et al.* 2007; Brown *et al.* 2013). The point in time when leaf primordium commitment travelling upwards on the apex collides with spikelet primordium moving downwards will determine the number of leaves to be produced. Thus, environmental conditions that control the formation of TS will ultimately determine the final number of leaves produced and the time of flowering. Following TS, committed leaves must appear in sequence followed by the ear and flowering, processes controlled predominantly by temperature alone (Weir *et al.* 1984; Ritchie & Otter 1985; Brooking *et al.* 1995; McMaster *et al.* 2008).

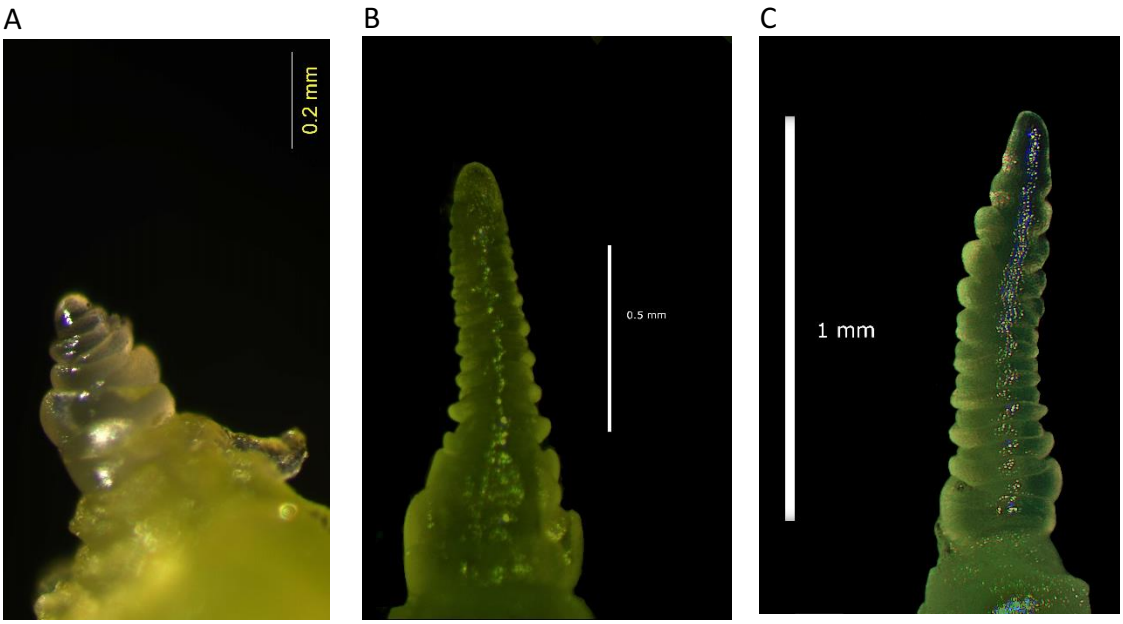


Figure 2.1. Visual references of the stem apex in the vegetative phase (a), elongated, early double ridge (b) and double ridge (c) for perennial ryegrass.

2.5 Factors controlling phenophase duration in perennial ryegrass

The sequential progression of an individual ryegrass plant through its phenophases completes the life cycle from seed to seed, provided conditions are non-limiting. The initial phase from sowing to emergence is conservative across cultivars (Moot *et al.* 2000). Post-emergence, vegetative development continues until the first sign of reproductive

development occurs. This is observed on the apex as it changes from the initiation of vegetative primordium (leaf production only) to a reproductive state. During this switch, DR is generally accepted as the first visual sign of commitment to reproductive development. At DR the apex has elongated, and in the middle portion of the apex the leaf and spikelet primordium are now the same size (Sweet *et al.* 1991) (Figure 2.1).

In most temperate perennial grasses, a sequential two step induction process is required to make the change from vegetative to reproductive growth (Heide 1994). Commonly plant's must experience cool temperatures and/or a short photoperiod, jointly referred to as vernalisation, before they will respond to the long photoperiod conditions required to induce flowering. Subsequently the effect of vernalisation is commonly referred to as 'primary induction' while the subsequent photoperiod response to trigger DR formation has become known as 'secondary induction' (Heide 1994; Aamlid *et al.* 2000).

The primary driver of crop development is the accumulation of temperature, referred to as thermal time (Tt) where generally, temperatures below or above an optimum, delay development. However in many species, including perennial ryegrass, low temperatures may be required to overcome a required period of cold chilling through vernalisation (Cooper 1960). Additionally, a short Pp may shorten the development phase during vernalisation but delay development post vernalisation (Cooper 1950). Thus, the first step in any examination of crop development is to define which phases are solely affected by temperature as accumulated thermal time, and which ones have specific photoperiod and/or vernalisation requirements. For modelling purposes, the use of accumulated Tt between two defined phases or stages often becomes a 'target' where a phase ends, or a stage is obtained, when the Tt target is achieved.

2.5.1 Temperature

Peacock (1975) showed that the stem apex was the critical site of temperature perception in *L. perenne*. Beddows (1968) demonstrated that the accumulation of soil temperature was closely related to apex development compared with standard air temperature. This suggests that the appropriate temperature measurements for any attempt to quantify development should be those which most accurately match the location of the apex, as

shown for wheat by Jamieson *et al.* (1995b). In crops with no Pp or vernalisation requirement, the most rapid completion of a life cycle occurs when temperatures are optimal. This is seen in crops such as maize which show minimal response to changes in photoperiod or vernalisation and hybrids are bred to match environments based on different total thermal time requirments. In other crops, such as wheat and perennial ryegrass, the genetic diversity from different germplasm accessions has resulted in a range of culitvars with different vernalisation and photoperiod responses.

In cultivars that are responsive to vernalisation and photoperiod, the phases of; emergence, stem extension, ear emergence and grain-fill are predominantly controlled by temperature (Weir *et al.* 1984; Jamieson *et al.* 1998b; Moot *et al.* 2000). For example, in the emergence phase, Moot *et al.* (2000) planted eight forage species over five sowing dates, ranging from 8 March to 8 May, and showed the time to emergence was conservative within a species when quantifited using accumulated temperature. For *L. perenne* this was 133°C days (T_b=2.1°C) while the number of days to emergence at least doubled as soil temperatures cooled.

For the stem extension and head emergence phases, no literature specific to any *Lolium* species was found. However, for wheat, both the CERES and ARCWHEAT1 models assume flowering occurs after a fixed target of accumulated Tt post TS (Weir *et al.* 1984; Ritchie & Otter 1985; McMaster *et al.* 2008). Brooking *et al.* (1995) demonstrated that for wheat, the duration from final leaf emergence to flowering was equivalent to three phyllochrons when using canopy temperature.

In *Lolium,* data have been presented for estimating the time from DR to ear emergence effectively incorportating the phases of ear development, stem extension and ear emergence. Keatinge *et al.* (1979) and Hurley *et al.*, (2006) suggested that the accumulated temperature between DR and ear emergence was not different for four and eight cultivars of perennial ryegrass respectively, at between 465 and 534°C days. However, these studies were completed at single locations with single sowing dates and identified a single critical Pp for reproductive initiation. A single critical Pp makes no reference to how Pp influences the rate of development and could alter the Tt duration

from DR to TS. Therefore, these results must be treated with caution. Additional information is required to identify when TS occurs in perennial ryegrass under different Pps.

For the seed filling phase, Chynoweth and Moot (2017) found the time from flowering to physiological maturity was conservative when described using air temperature alone, where 517°C days was required for three perennial ryegrass cultivars, sown on two dates and treated with three rates of Moddus® plant growth regulator. Similar results have been shown for other monocotyledonous species including, wheat (Loss *et al.* 1989) and oat (*Avena sativa*) (Crampton 1998).

The rate of progress through a plants life cycle can be delayed by a vernalisation and/or photoperiod requirement. These can be quantified by the impact on the final number of leaves on a single stem before inflorescence emergence, because leaf production is the mechanism by which plants delay the time to flowering. The response is observed as a delay between the emergence and TS stages, which cannot be quantified solely by the accumulation of temperature.

2.5.2 Vernalisation.

For genotypes that have a vernalisation response, low temperatures enable continued progression toward flowering and this has commonly been referred to as primary induction in recent literature (Heide 1994; Aamlid *et al.* 2000; Abel *et al.* 2017). Specifically, vernalisation relates to an exposure to cool temperatures and/or short Pp and is expressed by the influence on the primary induction phase. Langer (1979) stated that vernalisation of *L. perenne* was a facultative process where the longer a genotype is exposed the earlier it will flower, until the response is saturated (V_{sat}). Thus, V_{sat} occurs at the shortest duration to flowering and the final number of main stem leaves (FLN) is minimized. Many authors suggest *L. perenne* has an obligate (must have) requirement before initiating reproductive development (Cooper 1960; Evans 1960; Cooper & Calder 1964; Heide 1994) while others in the *Lolium* genus have no vernalisation requirement. Vernalisation is commonly expressed as a delay in progression compared with those genotypes with no requirement. Aamlid *et al.* (2000) demonstrated that exposure to 6°C/8 hr Pp reduced the number of days to 50% heading for the cultivar 'Veyo' (originating

from Italy) which showed no requirement for vernalisation to achieve flowering. Thus, while vernalisation was not required for flowering, it did influence the time to flowering. However, Aamlid *et al.* (2000) did not quantify the number of main stem leaves produced, making it difficult to interpret vernalisation effectiveness at different temperatures. These data were supported by Silsbury (1964) who showed a facultative response in four Australian *L. perenne* cultivars that achieved 50% flowering with no cold exposure, but all demonstrated a reduced final number of leaves following vernalisation.

Aamlid *et al.* (2000) also demonstrated cultivars 'Falster' and 'Kleppe' had an obligate requirement with a minimum of six weeks vernalisation before the onset of reproductive development (once transferred into long Pp). These results are consistent with Evans (1960) who showed a five week requirement for the cultivar 'S.24'. Furthermore Cooper (1950) planted different ryegrass species under both autumn field conditions and in a glass house under 24 h lighting (long day conditions). Annual types produced seed heads under both growing conditions. However, in *L. perenne,* few plants produced seed heads under glasshouse conditions. When measured 18 months after sowing, most plants were still only producing leaves and some had produced ~45-50 leaves compared with the 13-22 leaves produced before heading when sown outdoors in the autumn.

2.5.2.1 Effective temperatures and Pp for vernalisation

Cooper (1957) showed that the maximum effective temperature for vernalisation of perennial ryegrass was 10°C, while Evans (1960) showed the rate of vernalisation was quicker at 10°C compared with 4°C when using cultivars from the United Kingdom (UK) and New Zealand (germplasm likely originated from the UK). Under constant illumination (24 h) no flowering occurred above 14°C. This may set the upper limit for vernalisation. The influence of long Pps reducing the effectiveness of vernalisation in some genetics, at temperatures below 18°C, was reported by Cooper (1960) and Evans (1960) where temperatures of below 6°C were required in Pps longer than 16 h. However, in Pps of 8 h, 10-12°C was sufficient to induce flowering at appropriate durations. When plants were exposed to fluctuating temperature of 7°C night time and 20°C day time, vernalisation was saturated and hence flowering occurred. Heide (1994) stated that as temperatures increase, induction becomes increasingly dependent on short days until approximately

12-18°C, when primary induction cannot occur. Thus, it appears that temperature and Pp interact in a similar way to that proposed for wheat, by Brown *et al.* (2013), where as Pp increased the target required to induce a response increases, while cooler temperatures and/or increased duration are required to achieve the increasing target.

Cooper (1960) suggested that short days can fully substitute for cold temperatures when five cultivars reached 100% of plants heading following exposure to 12 weeks of 8 h Pp in a warm glasshouse followed by transfer to continuous lighting. In contrast, in an earlier experiment only 20% of plants headed when exposure to 8 weeks or 8 h Pp in a warm glasshouse, mean of five cultivars (four in common) suggesting a quantitative response or temperature differences between experiments (temperature data were not reported).

2.5.2.2 Temperature summary

The data presented have shown vernalisation requirements for completion of primary induction differ from obligate and large to facultative and intermediate depending on genotype and centre of origin. Thus, temperature and Pp interact to complete primary induction in *L. perenne*.

The mechanisms require further investigation with authors commonly only reporting flowering and days to heading without reporting influences on the number of leaves produced on the main stem, thus determining the time of V_{sat} is difficult. In addition, information at fixed temperatures and durations that saturate the reduction in the final number of main stem leaves would allow vernalisation functions to be developed for modelling.

2.5.3 Photoperiod

In addition to temperature, photoperiod can also alter the duration of primary and secondary induction phases in some genotypes. Perennial ryegrass is a quantitative long day species which means flowering can be delayed by days below a critical Pp. Cooper (1950) stated that the change from leaf to spikelet formation was a response to increasing photoperiod in the spring when the threshold value required by late season cultivars was longer compared with that of earlier flowering types. Aamlid *et al.* (2000) showed that the rate of development was accelerated by increasing photoperiod following

vernalisation in five European *L. perenne* cultivars, but did not quantify this through reductions in the final number of main stem leaves. Many authors have produced estimates of a 'critical' Pp required for the initiation of reproductive development. For example, Aamlid *et al.* (2000) used a criteria of 50% of plants flowering to show that critical Pp differed with genotype from between 12-14 h in 'Veyo' and 'Baca' respectively, up to 17 h in 'Falster' and 'Kleppe'. The authors commented that the rate of development was accelerated by increasing Pp (presented graphically as fewer days to heading) for all cultivars but they did not present this as rate of development. Additionally, Hurley *et al.* (2006) dissected the stem apex of eight European cultivars grown over winter in the field and showed critical day lengths differed from 12.7 to 15.0. Further to a critical Pp for induction, some variation in genotype response exists for the number of long day cycles before flower initiation (Heide 1994).

However, in many studies the 'critical Pp' is a measure of the Pp required to obtain flowering *per se* and does not represent a Pp response which can reduce the time to flowering. Therefore, it is probable that a 'critical Pp' ends the secondary induction phase and a facultative response exists during the ear development phase when the time to flowering is reduced as the Pp lengthens (Figure 2.2).

For this thesis, photoperiod is defined as including civil twilight from when the sun is 6° below the horizon as this period provides adequate light for photoperiod perception (Francis 1970; Weir *et al.* 1984; Hodges & Ritchie 1991).

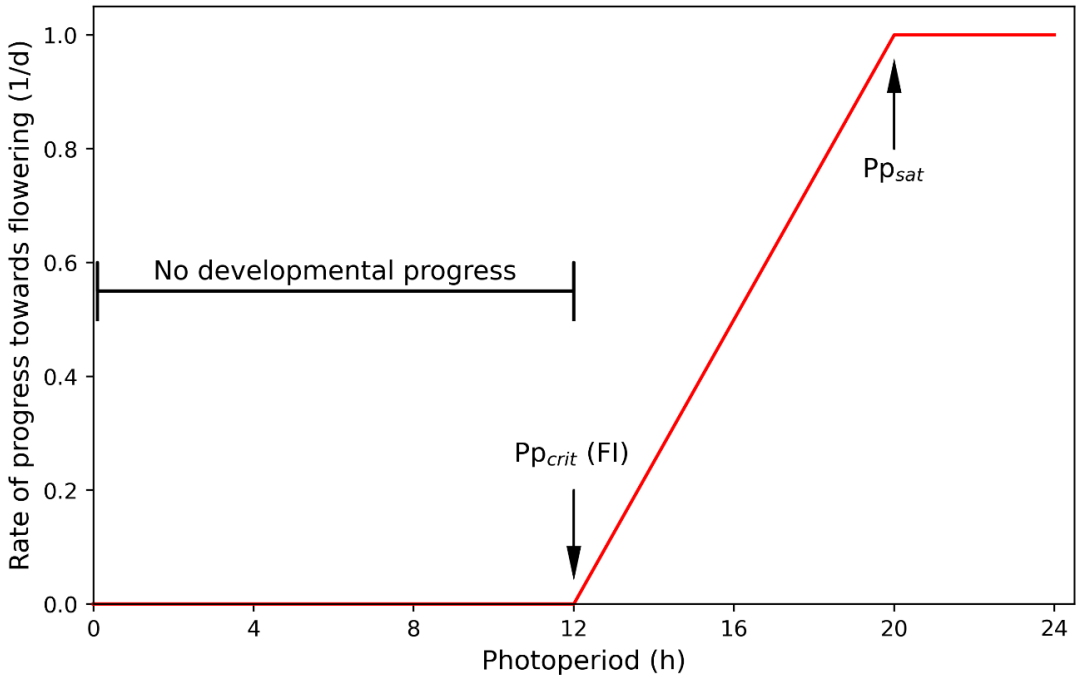


Figure 2.2. Theoretical function of how photoperiod (Pp) following floral initation (FI), minimum Pp for FI is Pp_{crit}, may impact the rate of progress toward terminal spikelet formation where unity (rate = 1) is the shortest duration possible.

2.6 Modelling phenology in wheat

The phenological response of crops to their environment can be quantified using equations. These can then be combined into crop simulation models that estimate crop growth and development. Mechanistic models attempt to describe the biological processes involved in crop growth and development by predicting when different developmental stages occur based on responses to environmental signals. These models usually calculate the time of flowering, based on thermal time (Tt) accumulation with a response to photoperiod and/or vernalisation quantified where the inverse of time to flowering is then the average rate of development.

Two of the most widely used modelling methods of wheat development are reviewed below with the expectation that these can be modified to represent perennial ryegrass. A third approach is briefly introduced which could further enhance the understanding of ryegrass development. All models contain empirical sub models that use Tt to track developmental progress where the Tt duration of a developmental phase becomes a Tt target. Tt is the accumulation of daily temperatures above a base temperature (T_b) below which development ceases.

The first approach (ARCWHEAT1) uses thermal time targets, modified by sub models representing vernalisation and Pp which restrict development through phases that are separated by the FI, DR and TS stages. Flowering then occurs after a fixed amount of photo-Tt accumulation post TS (Weir *et al.*, 1984). The second (*Sirius*) approach predicts the time and rate of primordium accumulation on the stem apex along with the number of emerged leaves and predicts the number of main stem leaves in response to Pp and vernalisation. Again flowering occurs after a set accumulation of Tt following the emergence of the final leaf (Jamieson *et al.* 1998b).

2.6.1 The Photo-vernal-thermal approach used in ARCWHEAT1 (Weir et al., 1984),

Within the crop lifecycle there are four Tt calculations used in the developmental sub model. Tt is calculated using eight sub contributions from a sinusoidal function between the maximum and minimum daily temperatures where:

$$\boldsymbol{Tt} = \frac{\mathbf{1}}{\mathbf{8}}\sum_{\boldsymbol{r=1}}^{\boldsymbol{r=8}}(\mathbf{T_H} - \mathbf{T_{base}}) \qquad \text{Equation 1}$$

where $$\mathbf{T_H} = \mathbf{T_{min}} + \boldsymbol{fr}\,(\mathbf{T_{max}} - \mathbf{T_{minH}}) \qquad \text{Equation 2}$$

and $$\boldsymbol{fr} = \frac{\mathbf{1}}{\mathbf{2}}[\mathbf{1} + \mathbf{cos}\frac{\mathbf{90}}{\mathbf{8}}(\mathbf{2}\boldsymbol{r} - \mathbf{1})] \qquad \text{Equation 3}$$

where T_H, T_b, T_{min} and T_{max} are in °C and negative values are treated as zero.

The optimum temperature is 26°C, above which the contribution drops to zero at 37°C. The four calculations of Tt are:

1. For the phase from sowing to emergence, Tt is calculated using a T_b of 1°C where the Tt target is 148°C days,
2. For the phase from emergence to DR, Tt is modified by vernalisation and photoperiod factors with a Tt target of 284°C days, Tt calculated with a T_b of 1°C,
3. The phase from DR to flowering has a Tt target of 600°C days (T_b of 1°C), modified by photoperiod only,
4. From flowering to maturity, Tt is calculated assuming a base temperature of 9°C with a target of 240°C days. More recently, 750°C days with a T_b of 0°C is used (Jamieson *et al.* 1998b).

Thus, the rate of progress from one developmental stage to the next is a function of accumulating temperature, modified by photoperiod and vernalisation where:

$$f = Tt \times P \times V$$

where: Tt= thermal time,
P = photoperiod factor (0 - 1) and,
V = vernalisation factor (0 = no vernalisation, 1 = fully vernalised).

2.6.1.1 Vernalisation function

The vernalisation function incorporates methods adjusted from Chujo (1966) and Lumsden (1980). Chujo (1966) suggested optimum temperatures for vernalisation in winter wheat ranged from approx. 4-12°C while temperatures of 1°C and those above 12°C were less effective. Lumsden (1980) reanalysed the data presented by Chujo (1966) to examine the developmental responses to vernalisation and proposed a model incorporating a minimum period, or lag phase, of eight vernal days (V_b = 8), a period of linear response to vernalisation which lasts 33 days, until saturation (V_b until V_{sat}, V_{sat} = 41 days) followed by a plateau where developmental rate was not increased by additional vernalisation (Figure 2.4).

Based on this, the vernalisation function (V) that quantifies developmental progress in ARCWHEAT1 is represented by a variable ranging between 0 and 1 in response to the accumulation of vernal degree days (*Vdd*).

The vernalisation function is represented by four cardinal temperatures where T1 = -4°C, T2 = 3°C, T3 = 10°C and T4 = 17°C (Figure 2.3). Thus, the effectiveness of temperature on vernalisation (*Veff*) is:

$$\begin{aligned} Veff &= 1 && \text{If: } 3°C < T < 10°C \\ Veff &= \frac{T - -4°C}{3°C - -4°C} && \text{If: } -4°C < T < 3°C \\ Veff &= \frac{17°C - T}{17°C - 10°C} && \text{If: } 10°C < T < 17°C \end{aligned} \qquad \text{Equation 4}$$

where T is the current temperature.

The mean *Veff* value is calculated each day from the temperature curves and the accumulated vernal degree days (*Vdd*) summed from sowing.

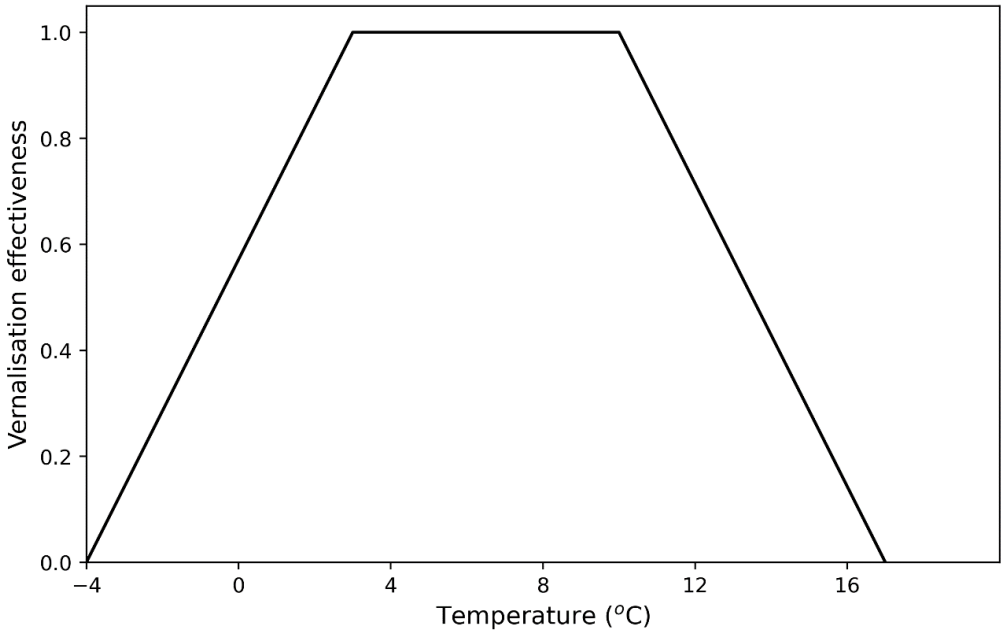


Figure 2.3. Graphical representation of the cardinal temperatures used to model vernalisation in ARCWHEAT1.

The function c(V) used to modify Tt, represented graphically in Figure 2.4, is calculated from the accumulated *Vdd* where:

c(V) = 0	If: $Vdd < V_{base}$	
$c(V) = \frac{Vdd - V_{base}}{V_{sat} - V_{base}}$	If: $V_{base} < Vdd < V_{sat}$	Equation 5
c(V) = 1	If: $Vdd > V_{sat}$	

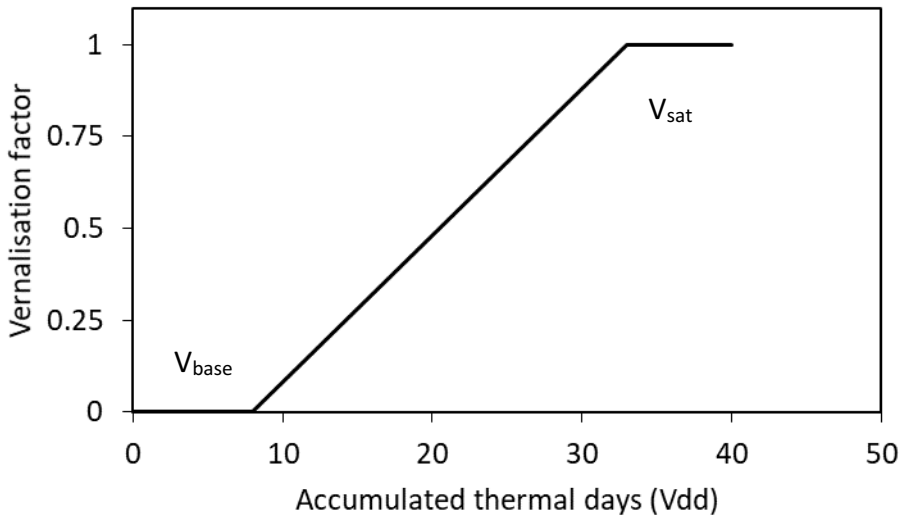


Figure 2.4. Graphic representation of the accumulated vernalisation factor used to restrict development in the ARCWHEAT1 model. For example, there are 8 base *Vdd*, 33 *Vdd* then saturation is achieved at 41 *Vdd*.

2.6.1.2 Photoperiod function

Pp effectiveness ranges from 0 to 1 and is based on the number of effective hours calculated each day. This is calculated above the base Pp as the daily Pp divided by the optimum Pp (Weir *et al.* 1984). The Pp function is based on extrapolation of data from the literature by Lumsden (1980) who proposed a linear method based on a saturating Pp (Pp_{opt}) and base Pp (Pp_b). Lumsden (1980) set a saturating Pp at 20 hours based on data from Holmes (1973) and Rahman and Wilson (1977) who both showed no increase in rate of development at longer Pps. Lumsden (1980) estimated Pp_b by plotting the rate of development corrected for temperature and vernalisation against 'mean effective photoperiod'. Following extrapolation of the data a Pp_b of 0 hours was used until double ridge and 7 hours following double ridge. In practice Pp_b is set by the minimum Pp at the field location and Pp_{opt} is the maximum Pp, which is usually less than 20 hours so is always causing a delay in development. Following TS, the Pp factor is 1 i.e. non-limiting.

In ARCWHEAT1 the photo-thermal time increment is found by multiplying the thermal time function by the photoperiod factor, P where;

$$
\begin{cases}
\mathrm{P} = 0 & \text{If: } Pp < P_{base} \\
\mathrm{P} = \dfrac{Pp - P_{base}}{P_{opt} - P_{base}} & \text{If: } P_{base} < Pp < P_{opt} \\
\mathrm{P} = 1 & \text{If: } Pp > P_{opt}
\end{cases}
\qquad \text{Equation 6}
$$

where Pp = current Pp, Pp_{opt} = 20 hours, Pp_{base} = 0 hours until DR followed by 7 hours DR to TS.

P_{opt} is the photoperiod beyond which development is not sensitive to Pp and following TS the Pp factor is 1 i.e. non-limiting. By multiplying daily Tt, by factors for vernalisation and Pp, estimates of developmental stages are represented by Tt targets. As both the vernalisation and Pp functions limit Tt accumulation, they delay modelled crop development. Differences in genotypes are simulated through vernalisation and Pp sensitivity coefficients that alter responses to climatic conditions. Differences in intrinsic earliness are modelled by adjusting the Tt targets. ARCWHEAT1 assumes the thermal time durations are independent from each other, however Jamieson *et al.* (2007) showed this to be incorrect.

2.6.2 Final leaf number approach

The *Sirius* approach to modelling development differs from ARCWHEAT1 and most other models where differences associated with vernalisation and Pp are described mechanistically by adjusting the number of primordium initiated, leaves produced, and the final number of main stem leaves (Jamieson *et al.* 1998a). The number of days to flowering is a function of the number of main-stem leaves produced (FLN) and the time required for each leaf to appear (phyllochron), followed by the duration from the appearance of the final leaf until flowering (anthesis) (FL-AN) where:

$$Days\ to\ Anthesis = FLN \times \frac{phyllochron}{Mean\ Temp} + \frac{FL - AN}{Mean\ Temp} \qquad \text{Equation 7}$$

Both the phyllochron and FL-AN are decreased with increasing temperature and can be treated as Tt accumulation targets. The environmental factors of temperature and genotype coefficients that control FLN, phyllochron and FL-AN combine to influence flowering date. However, FLN, phyllochron and FL-AN interact with vernalisation and photoperiod directly. For example, a spring type photoperiod insensitive wheat will produce the same FLN regardless of environmental conditions. In contrast a photoperiod sensitive spring type will increase its FLN in response to a shorter photoperiod. A winter type wheat will have a higher FLN than a spring type when raised under warm conditions but its FLN can reduce to the same level as a spring type wheat if it encounters sufficient cool temperature early in its development (Brooking & Jamieson 2002).

Sirius predicts final leaf ligule appearance based on the rate of leaf appearance and final main stem leaf number in response to photoperiod (Brooking *et al.* 1995) and vernalisation (Brooking 1996; Robertson *et al.* 1996).

Current leaf number (LN_c) is modelled as a function of leaf primordium initiation and base phyllochron for each cultivar ($Phyll_{base}$). Thermal time is calculated above a base temperature of 0°C based on apex temperature. Initially apex temperature is assumed to be that of near soil surface (0-2 cm) calculated from canopy temperature and the surface energy balance (Jamieson *et al.* 1995b) and then canopy temperature after HS4. Phyllochron follows a segmented linear model where the first two leaves appear at a 75%

of $Phyll_{base}$, leaves 2-8 appear at $Phyll_{base}$, and all subsequent leaves appear at 140% of $Phyll_{base}$ (Equation 8).

$$LN_c = \begin{cases} 0.75 \times Phyll_{base} \times Tt & LN < 2 \\ Phyll_{base} \times Tt & 2 \leq LN < 8 \\ 1.4 \times Phyll_{base} \times Tt & LN \geq 8 \end{cases} \quad \text{Equation 8}$$

The photoperiod and vernalisation sub models require the calculation of the number of vegetative primordium which is calculated directly from the current HS as the number of vegetative primordium and leaves are coordinated (Kirby 1990). Prediction of vegetative primordium number can be given by Equation 9.

$$P = 2LNc + 3 \quad \text{Equation 9}$$

Where P is primordium number
LN_c is the current leaf number or Haun stage

Equation 9 can be used to calculate leaf stage at any desired primordium number or vice versa.

Vernalisation is accounted for where the accumulated exposure to cool temperatures reduces the number of leaves produced on a main stem (Robertson *et al.* 1996). The vernalisation function is optimum (T_{Vopt}) at 8°C and decreases linearly to 0°C (T_{Vmin}) and 15°C (T_{Vmax}). The effect of vernalisation on the final number of leaves initiated on the shoot apex, at the time of saturation (V_{sat}), is a function of the duration to saturate vernalisation and the rate of leaf primordium initiation during that time. Added to this is the number of leaf primordium present in the embryo of the seed. For example, the rate of leaf primordium production (R_{leaf}), is assumed to be a linear function of temperature (Hay & Kirby 1991), and can be estimated by:

$$R_{leaf} = Tv \times P \quad \text{Equation 10}$$

Where: P is the rate of leaf primordium initiation per °C day between 0°C and 20°C
T_v is the vernalising temperature

The number of leaves (N_{leaf}) initiated on the main stem can then be estimated by

$$\mathrm{N}leaf \;\; = \left(\mathrm{R}_{leaf} \times \mathrm{D}\right) + 3 \qquad \text{Equation 11}$$

Where: R_{leaf} is the rate of leaf primordium initiation,
D is the duration required to saturate vernalisation at a known constant temperature,
3 a constant representing the number of primordium initiated prior to germination (present in the seed).

If the duration of vernalisation is known at multiple temperatures, the rate of saturation of vernalisation can be obtained from the inverse of the duration, and cultivar specific coefficients of the linear relationship can be obtained. The daily vernalisation rate (VAI, slope of the rate of saturation regression) increases at a constant rate with daily mean soil or canopy temperature from its base value at T_{Vmin} (VBEE, the intercept of the rate of saturation regression).

$$\begin{aligned} \mathrm{V}_{rate} &= \mathrm{VAI} \times \mathrm{T} + \mathrm{VBEE} && T_{Vmin} \le T \le T_{Vopt} \\ \mathrm{V}_{rate} &= \left(\mathrm{VAI} \times T_{Vopt} + \mathrm{VBEE}\right) \times \frac{(T_{Vmax} - T)}{(T_{Vmax} - T_{Vopt})} && T_{Vopt} < T \le T_{Vmax} \\ \mathrm{V}_{rate} &= 0 && T_{Vmin} > T \\ & && or \;\; T > T_{\,Vmax} \end{aligned} \qquad \text{Equation 12}$$

The progress toward V_{sat} is the sum of daily V_{rate}, where a value of 1 is V_{sat}.

$$V_{prog} = \sum_{day=1}^{n} V_{rate} \qquad \text{Equation 13}$$

Leaf number is defined by two cultivar specific parameters, absolute minimum (FLN_{min}) and absolute maximum (FLN_{max}) number of leaves on the main stem (He *et al.* 2012). For cultivars requiring vernalisation, the model starts by assuming the potential leaf number is FLN_{max} and FLN reduces as the vernalisation function increases (Equation 14).

$$FLN = FLN_{max} - (FLN_{max} - FLN_{min}) \times V_{prog} \qquad \text{Equation 14}$$

Vernalisation is considered complete when either the vernalisation function reaches 1, the FLN reduces to the cultivar specific minimum (approx. 8) or the number of leaf primordium increases to reach the decreasing FLN.

In *Sirius*, the crop responds only to Pp after V_{sat} occurs. In long Pp, the FLN is set by the number of leaf primordium set when V_{sat} is complete. If the Pp at V_{sat} is short, the FLN increases as per spring wheat. In spring types, the potential FLN starts out equal to FLN_{min} as V_{prog} equals 1 at sowing, thus FLN = FLN_{min}. However, when short Pp are experienced the number of leaves added increases to delay flowering by a linear function from 0 leaves added above 16 h Pp to a maximum below 8 h Pp.

Final number of leaves for spring wheat (or winter wheat following V_{sat}) can be given by:

$$FLN = \begin{cases} LN_{min} + b\,(D_{sat} - D) & D < D_{sat} \\ LN_{min} & D \geq D_{sat} \end{cases} \qquad \text{Equation 15}$$

Where: LN_{min} is the minimum possible leaf number
b is the slope of the day length response regression (commonly 0.3-0.5)
D_{sat} is the saturating day length
D is the current day length

FLN is recalculated daily from Haun 1.5 until final leaf number is set providing an accurate prediction of final number of leaves. The leaf number added is determined when the current Pp at two leaves after the final leaf primordium has formed (Brooking *et al.* 1995) or at terminal spikelet formation (Brown *et al.* 2013).

A potential issue with the *Sirius* approach is that it requires a maximum number of main stem leaves for the final leaf number calculation. In some perennial ryegrass genotypes, the potential leaf number is infinite unless vernalisation occurs. Thus, the maximum leaf number could be set to a large number which would prolong the duration to flowering, unless the vernalisation function becomes saturated.

2.6.3 Model comparisons

The approach used in ARCWHEAT1 is relatively simple and allows users to describe cultivars as vernalisation or photoperiod sensitive and to develop cultivar specific parameters to allow for maturity differences. ARCWHEAT1 has limitations in that it predicts vernalisation and Pp responses concurrently when in perennial grasses these are reported as two sequential events (Heide 1994). While *Sirius* treats vernalisation and Pp responses concurrently it does not account for short day vernalisation under warm conditions. Neither model currently allow for the use of a base Pp for secondary induction (FI) independent of vernalisation, e.g. *Sirius* uses a regression response and ARCWHEAT1 treats the time to DR as a multiplicative vernalisation and Pp response. The advantage of *Sirius* over ARCWHEAT1 is that it provides a more complete understanding of the relationship between leaf appearance and reproductive development, thus providing an enhanced understanding of the underlying processes occurring during phenological development. Additionally, if *Sirius* can predict leaf appearance, and leaves are associated with nodes, estimates of crop growth stages can be made for management of crop inputs e.g. nitrogen and fungicide input.

2.6.4 A molecular model

A more recent model proposed by Brown *et al.* (2013) suggested linking molecular and physiological models to predict the time of final leaf emergence based on the HS at which TS occurs. The current HS is calculated using a similar method to *Sirius* (Equation 8) but the base phyllochron was changed to 120°C days, where emergence takes 0.9 phyllochron and break points for the change in phyllochron were adjusted to 2.5 and 7 HS. The major difference from *Sirius* is that apparent gene expression of the four major vernalisation genes known to occur in wheat (Trevaskis *et al.* 2007) are used to determine the HS at which TS occurs, from which, the final number of leaves is calculated by Equation 16.

$$FLN = 2.86 + 1.1 \times TS^{HS} \qquad \text{Equation 16}$$

Where: TS^{HS} is the Haun stage at which terminal spikelet formation occurs

For TS formation to occur, the *VRN1* gene and subsequently the *VRN3* gene, require upregulation. Apparent gene expression is calculated relative to changes in HS to account

for the effects of temperature *per se* on leaf number. *VRN1* expression occurs at a base rate or either 0.08/HS or 0.17/HS for winter or spring types respectively suggesting V_{sat} will occur at some future point. *VRN1* has a target relative expression of 0.76 for V_{sat} to occur. *VRN1* is upregulated by exposure to low temperatures while *VRN4* represses *VRN1* under high temperatures. Following V_{sat}, *VRN3* is upregulated and apparent expression begins, however *VRN3* is suppressed by *VRN2* under long Pp, requiring further *VRN1* upregulation to promote *VRN3* under such conditions. As such the target *VRN1* of 0.76 for V_{sat} occurs in 8 h Pp conditions and increases at 0.026/HS under 16 h Pp. *VRN3* is expressed after V_{sat} (*VRN1* target achieved) with apparent expression rates increasing from 0.15/HS at 8 hr Pp to 0.33/HS at 16 hr Pp. When *VRN3* apparent is equal to 1, terminal spikelet formation occurs and the final leaf number calculated from Equation 16. The time from terminal spikelet to final leaf emergence is calculated as the number of leaves to emerge multiplied by the phyllochron. The time to flowering and maturity can then be estimated as per *Sirius*.

The advantage of ARCWHEAT1 and the model proposed by Brown *et al.* (2013) over *Sirius* is that they do not require a cultivar specific maximum FLN. With apparent gene expression the accumulation of *VRN1* can be set to 0, thus prolonging flowering indefinitely in species with an obligate requirement for cold conditions. Secondly this process provides a role for each of the known flowering time genes in wheat, enhancing the linkage between physiological and molecular processes. In ryegrass, a homologue of the wheat *VRN1* gene has been identified as *LpVRN1* (Jensen *et al.* 2005), while *LpFT3* is likely to replicate the activity of *VRN3* and promote flowering (Gagic 2007; Wang & Forster 2017). *VRN3* in wheat and barley (*Hordeum vulgare*) is an orthologue of a FLOWERING LOCUS T (FT) gene (Yan *et al.* 2006). *LpFT3* is controlled by a number of genes from the CONSTANS (CO) (Pp response) and GIGANTEA (GI) (circadian clock) gene groups (Gagic 2007) which could act as *VRN2*. An active *L. perenne VRN2* gene has not yet been identified (Wang & Forster 2017), although a similar vernalisation response as wheat occurs in perennial ryegrass where vernalisation in short days occurs faster than in long days. Thus, it appears perennial ryegrass contains genes that function with similar mechanisms as *VRN1*, *VRN2*, and *VRN3* suggesting similar apparent gene expressions could be developed for perennial ryegrass.

It is expected that ecotypes of perennial ryegrass will differ from being comparable to photoperiod sensitive spring wheat through to winter type wheat and this research aims to develop a model that can describe these responses for different perennial ryegrasses.

2.7 Conclusions

- Perennial ryegrass has a wide natural range and demonstrates responses to Pp and temperature that appear related to the latitude of origin.
- Perennial ryegrass follows a predetermined sequence of phenophases from germination to seed maturity.
- Following emergence, perennial ryegrass maintains a vegetative state until primary and secondary induction phases are competed when floral initiation occurs.
- The duration of primary induction is influenced by temperature while Pp may influence the duration of both primary and secondary induction.
- Crop models use a range of approaches to quantify crop responses to temperature and Pp that allow the prediction of developmental stages.
- ARCWHEAT1 uses thermal time targets, modified by vernalisation and Pp to restrict development while the *Sirius* approach predicts the number of main stem leaves in response to Pp and vernalisation.

3 GERMPLASM SCREENING

Objective 1

- To identify germplasm with a range of responses to vernalisation, photoperiod and temperature treatments.

Null hypothesis:

- All genotypes will have the same temperature and photoperiod requirements to induce reproductive development.

3.1 Introduction

Many of the important forage grasses, including perennial ryegrass, are reported to have a dual requirement to achieve floral initiation (FI) where primary induction (PI) is achieved via vernalisation followed by exposure to a long photoperiod where secondary induction triggers FI (Heide 1994). Cooper (1951) showed there were three environmental conditions controlling spikelet initiation of perennial ryegrass; (i) low temperature and/or, (ii) short days, usually followed by (iii) long days. Cooper (1950) demonstrated that when perennial ryegrass was grown under glasshouse conditions, only a few plants produced seed heads under continuous lighting. Up to 18 months after sowing most plants were still producing leaves only, with some up to the 45-50th leaf stage compared with the 13-22 leaves produced before heading when sown outdoors in autumn. These results suggest that perennial ryegrass genotypes must be exposed to winter conditions of low temperatures, and/or short days, before they can respond to increasing spring photoperiods i.e. they have an obligate vernalisation requirement. Aamlid *et al.* (2000) showed there is variation in the primary induction phase between genotypes from different origins where one flowered with little or no exposure to vernalising conditions while others required 12 weeks of exposure at 8°C. McCormick *et al.* (2014) showed some plants of the perennial ryegrass cultivar, 'Grasslands Samson' produced seed heads with no exposure to vernalising conditions (grown at 18-19°C and 20 h photoperiod) but the optimum response, based on the reduction in the number of main stem leaves, was after 6 weeks exposure to 4°C. Flowering after no exposure to cool conditions, as shown by Aamlid *et al.* (2000) and McCormick *et al.* (2014), demonstrates differences between genotypes of perennial ryegrass.

Through four controlled environment experiments, this chapter screens material sourced from different ecophysiological zones to identify genotype groups that exhibit different responses for the induction of flowering. From these, individual genotypes that represent each distinct group will be progressed into further, more detailed, experiments to quantify the impact of vernalisation and photoperiod in later chapters.

3.2 Materials and methods

All four experiments were completed in 'Conviron BDW' walk in controlled environment chambers at Lincoln University.

3.2.1 Germplasm and treatment conditions

For these screening experiments, up to 17 perennial ryegrass ecotypes and 5 commercial genotypes/ecotypes were sourced from the Margot Ford Germplasm Centre, located at AgResearch's Grasslands Campus, Palmerston North (Table 3.1). Ecotypes were selected to represent the range of latitudes and altitudes within the natural distribution of perennial ryegrass, which includes Europe, Asia and North Africa (Terrell 1968).

In Experiment 1, 22 ecotypes were compared under constant 18°C and 20 h Pp (Table 3.2) to identify any genotypes that flowered without exposure to vernalising conditions. The expectation was that no flowering would occur. However, many plants flowered in Experiment 1, (Figure 3.4, Appendix 1), therefore Experiments 2 and 3 were used to compare 13 ecotypes grown at constant 18°C with either 14 or 11 h Pp, respectively. The reduced number of ecotypes in Experiments 2 and 3 was due to removing those with poor germination, vigour or similar responses to other ecotypes. A 'fresh' sample of 'Grasslands Nui' was collected from field grown seed and included as a reference to the 'Grasslands Nui' line sourced from the Margot Forde Germplasm Centre.

Experiment 4, included 22 ecotypes and investigated their vernalisation requirement. Genotypes were exposed to 4°C under constant darkness for five weeks (until emergence) followed by an additional seven weeks at 4°C under 20 h Pp. This was followed by constant 18°C and 20 h Pp. The 20 h Pp was chosen so that as plants were vernalised they experienced a long Pp stimulus which would immediately induce FI.

All experiments were replicated four times in a randomised complete block design inside a single controlled environment chamber.

Table 3.1. Perennial ryegrass (*Lolium perenne*) ecotypes or genotypes sourced for use from the Margot Forde Germplasm Centre, AgResearch, Palmerston North, New Zealand and their associated maximum and minimum photoperiods (Pp) at their origin (includes civil twilight).

#	Ecotype	Country of Origin	Lat	long	Altitude	Min Pp	Max Pp
1	A15364	Morocco	33	-5	1820	10.9	15.3
2*	A15361	Morocco	34	-4	1120	10.8	15.4
3	A15323	Algeria	36	5	50	10.7	15.6
4	A15371	Tunisia	37	9	237	10.6	15.7
5*	A14496	Turkey	41	41	2574	10.3	16.2
6*	A14499	Turkey	41	41	0	10.3	16.2
7	A15769	Portugal	42	-8	968	10.1	16.4
8*	A6012	Spain	43	-8	513	10.1	16.5
9	A7294	Spain	44	-8	9	10	16.7
10	A7283	Italy	46	11	114	9.7	17.0
11*	A14544	Italy	46	13	367	9.7	17.0
12*	A17173	Ukraine	48			9.6	17.4
13	A12434	Netherlands	52	5	10	9.1	18.4
14	A15338	Denmark	54	10	27	8.8	19
15	A15368	Sweden	58	13	50	8.2	20.7
16*	A17183	Norway	59	6	20	8.0	21.4
17*	A17184	Norway	60	5	10	7.79	22.4
18*	Kangaroo Valley Early	Australia				10.8	15.4
19*	Grasslands Nui	New Zealand				10.6	15.7
20*	Kleppe	Norway				8.0	21.4
21	Aurora	Switzerland				9.7	17.0
22*	Medea	Algeria				10.9	15.3
23*[1]	Grasslands Nui	New Zealand				10.6	15.7

* = ecotypes used under all photoperiod experiments

*1 = Fresh Grasslands Nui collected from harvested seed – i.e. no chilling in the Margot Forde Germplasm Centre.

3.2.2 Chamber conditions

For all experiments, the controlled environment chambers were set to constant temperature and Pp conditions (Table 3.2) with a target relative humidity of 70%, and a target light intensity of 700 µmol/m^2/s at pot level. At ≤8°C these chambers undergo a defrost cycle at approximately 3 hourly intervals, during which the main chamber lighting is turned off for ~15 minutes. In Experiment 4, at 4°C, supplementary LED lighting was

installed inside the chamber approximately 50 cm above the plants to supply ~120 µmol/m^2/s when the main chamber lighting was off. Light intensity was monitored at 0.5 second intervals using an Apogee SP110 Pyranometer monitored by a Campbell scientific CS1000 data logger. When light intensity was less than 100 W/m^2 (during daylight hours only), LED lighting was automatically switched on to maintain daylight conditions during the defrost cycles.

Temperature was recorded at hourly intervals in each chamber using a Hobo® U12 Outdoor/Industrial data logger equipped with four TMCx-HD temperature sensors. One sensor recorded air temperature inside a radiation shield at 100 mm above the pots, or plants, while three were placed at seed depth during sowing to record temperature at seed level.

Table 3.2. Temperature and photoperiod combinations used in controlled environment chambers to investigate flowering of perennial ryegrass at Lincoln University, New Zealand.

Experiment #	Pre-treatment		Main conditions	
	Temperature (°C)	Photoperiod (hours)	Temperature (°C)	Photoperiod (hours)
1	-	-	18	20
2	-	-	18	14
3	-	-	18	11
4	4	Dark f.b. 20[1]	18	20

[1] 5 weeks of darkness followed by 7 weeks at 20 hour photoperiod.

3.2.3 Sowing and establishment

Pots were 180 mm in diameter, contained ~3 litres of a 9 month potting mix (80% composted bark, 20% Pumice (1-7 mm), Osmocote® 15-9-12, Horticultural lime and Hydraflo®) and were placed in saucers on tables at ~1.2 m above floor level. For each experiment, eight seeds of each ecotype were placed directly on damp potting mix and covered with 20 mm of damp seed raising mix. Prior to seedling emergence, pots were top watered to ensure adequate moisture for germination. Following emergence pots were bottom watered. Pots were watered using standard tap water that was acclimatised in 20 L containers inside each controlled environment room for at least 24 hours. At 18°C watering was required at 1-2 day intervals. Each week all experiments were fertilised

using a half strength Hoagland solution (Epstein 1972). Following seedling emergence, when seedlings produced two leaf ligules, they were thinned by hand to four seedlings per pot.

3.2.4 Measurements

In 18°C chambers, emerged seedlings were counted at 1-2 day intervals until a constant seedling number was achieved on three successive measurements. The main stem was tagged using coloured electrical wires at the commencement of tillering and all subsequent assessments were on the main stem. The number of emerged main stem leaves was tracked by placing tags underneath the newest, fully emerged leaf to ensure they could not be dislodged. A leaf was considered fully emerged when the ligule was visible. Tags were added at approximately 2-3 leaf intervals. Where appropriate, from final leaf ligule visible, development was tracked by Zadoks growth stage (Zadoks *et al.* 1974), including time of spike emergence, and in some genotypes the onset of flowering.

3.2.4.1 Final assessment

At the final assessment, plants that had not reached final leaf emergence were dissected and the stem apex visually assessed to determine its status (vegetative, DR or TS etc). If double ridge could be confirmed, that plant was classified as reproductive. The number of leaves yet to emerge was counted and if the terminal spikelet was visible, the number of spikelets present was assessed. If TS was obtained, the leaves yet to emerge were added to the final number of leaves for that plant. Plants that were vegetative or yet to obtain TS were excluded from main stem leaf number calculations, as the final number of leaves could not be confirmed. For data analysis, treatments were reproductive if at least 50% of plants were at DR or beyond. For plants that had reached final leaf emergence the number of spikelet's present was counted on the main stem only. The length of the last fully emerged leaf on each main stem was measured from the ligule to the leaf tip.

Finally, genotypes were assessed as being morphologically representative of perennial ryegrass. Specifically, they were assessed for general leaf shape and structure along with the presence of awns on developing seed heads. Accession #1, 15364, was discontinued as the leaf and seed head characteristic were assessed as different from perennial

ryegrass. ‘Kangaroo Valley’ was not progressed as it displayed seed heads with awns present.

3.2.5 Statistical analysis

Data were collated in MS excel, interrogated utilising python and subjected to ANOVA using Genstat®19 (VSN 2019). For ecotypes where the latitude of origin was not available for analysis, latitudes were assigned based on reported breeding/collection history e.g. ‘Grasslands Nui’ was collected from pastures in Mangere, South Auckland, New Zealand and was assigned a latitude of 37°, but likely originated from England (~50°) with early European settlers.

Time to 50% emergence was analysed by converting the plants counted at each assessment to the percentage of final count. Logistic curves were fitted to each individual pot using the least squares regression method where the start point was constrained close to zero and the maximum set close to 100 (Equation 17). The inflection point (M) is equal to the time to 50% emergence. Curve parameters were subjected to one way analysis of variance (ANOVA) for individual experiments. To compare across experiments, treatment means were subjected to a randomised complete block ANOVA with a treatment structure of ‘ecotype + block’ for each parameter, where each experiment represented a block. For a chamber to be included in such analysis, a minimum of eight ecotypes must have data, e.g. in Experiment 2, 18°C 14 h, only two ecotypes were reproductive and thus was excluded from comparison in several parameters.

$$y = \frac{100}{1 + \exp\left(-B * (x - M)\right)} \qquad \text{Equation 17}$$

Where M is the inflection point (time to 50% emergence) and
B is a curvature parameter.

Phyllochron was calculated by first fitting linear regression (Equation 18) to the accumulated number of fully emerged leaves against accumulated thermal time for each ecotype using the “olm” (Ordinary Least Squares) method in the “statsmodels” package inside Python (Seabold & Perktold 2010). The phyllochron was subsequently estimated as one divided by the slope of the regression equation (Equation 19).

$$y = ax + c$$ Equation 18

Where a is the slope
b is the intercept on the y axis.

$$\frac{1}{a}$$ Equation 19

Where a is the slope from Equation 18

The final number of main stem leaves was calculated as the mean of all reproductive plants in each pot.

Differences among treatments were identified by Fisher's protected least significant difference test ($\alpha = 0.05$) ($LSD_{0.05}$) where the ANOVA P value was less than 0.05 (≤0.05). In text, the treatment means are presented as the mean ± the 'standard error of the mean' (SEM) as calculated by Genstat®19.

3.2.6 Temperatures

The measured daily mean air temperatures recorded inside all controlled environment chambers, at canopy height, were near the target temperatures. The mean daily potting mix temperature, at plant apex level, fluctuated with the duration of the Pp and the amount of canopy cover shading pots below. For example, in the 20 h Pp chambers, the potting mix temperatures were commonly ~2.5°C above air temperature due to the radiant heat emitted by the high intensity discharge lamps (Figure 3.1A,B), until the canopy shaded the surface. Following canopy closure mean pot temperature was commonly 1°C lower than air temperature.

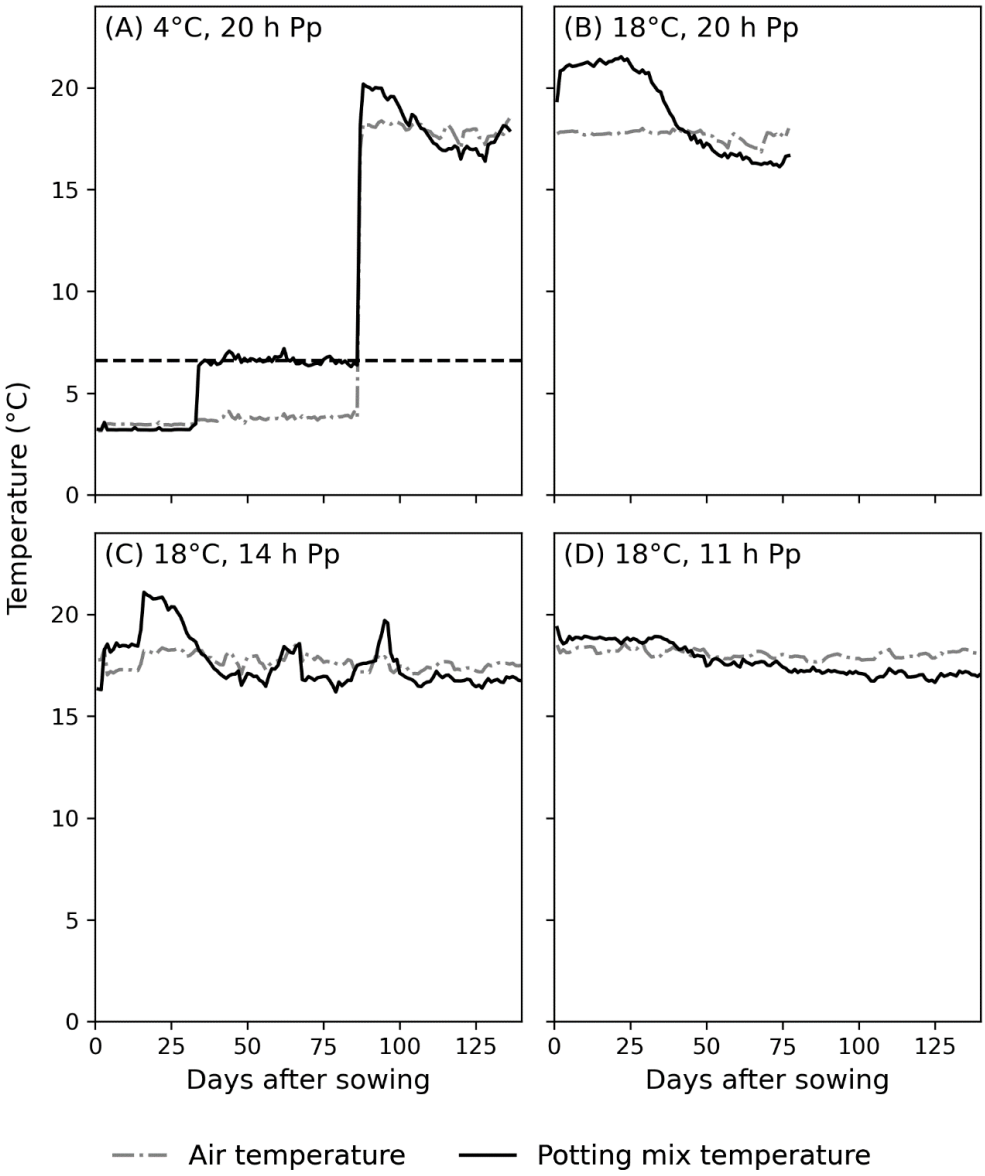


Figure 3.1. Air and potting mix temperature at 3 cm depth (stem apex depth) for four controlled environment chambers used to grow perennial ryegrass at Lincoln University, New Zealand. Dashed line in figure A = 6.6°C.

3.2.7 Thermal time calculation

Thermal time (Tt) was calculated from actual recorded temperature data as either the mean of the three soil probes for soil Tt (Tt_{soil}) or from the single shielded air temperature probe for air Tt (Tt_{air}). Calculations were made each hour by dividing the hourly temperature reading by 24 and summing the daily totals (Equation 20). No consideration was made for base temperature (T_b) as chambers ran at constant temperatures above those considered close to T_b for ryegrass e.g. 0 - 1.4°C (Moot *et al.* 2000) and growth or development proceeded in all chambers. Thus, a T_b of 0°C was used for all calculations.

For time to emergence, Tt_{soil} was used as this reflects the temperatures experienced by the seed. Where soil temperatures deviated from air temp e.g. in the 20 h Pp chambers, (Figure 3.1) Tt_{soil} was used until canopy closure when Tt_{air} was used to represent temperatures at the stem apex (Jamieson *et al.* 1995b).

$$\text{Accumulated Tt} = \sum_{i}^{0} Tt \text{ daily} = \sum_{n}^{0} \left(\frac{T}{24}\right) \qquad \text{Equation 20}$$

Where T is the hourly temperature recorded
i is the number of days after sowing and
n is the number of hours passed within the current day

3.3 Results

3.3.1 Seedling emergence

When expressed in days, seedling emergence was constant at 6.08 (± 0.94) days for all 18°C chambers but was delayed (P<0.001) to 34.5 days when germination occurred at 4°C (Figure 3.2). Time to 50% emergence was constant in thermal time between ecotypes (P=0.189) and experiments (P=0.113) at 123°C days (±2.21 °C days) (Figure 3.3).

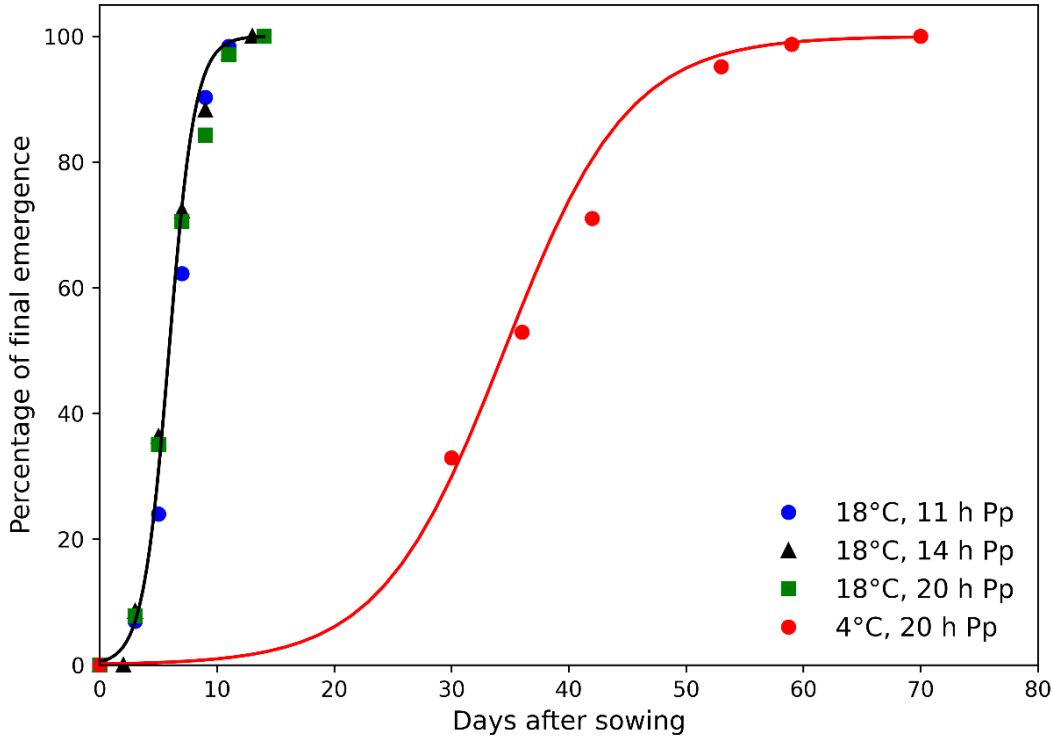


Figure 3.2. Emergence percentage of perennial ryegrass grown under four temperature and photoperiod regimes in controlled environment chambers at Lincoln University, New Zealand. Each data point is the mean of either 12 or 22 individual ecotypes replicated four times. At 18°C, y = 100/1+exp(-0.93×(x-6.06)), at 4°C, y = 100/1+exp(-0.18× (x -34.5)).

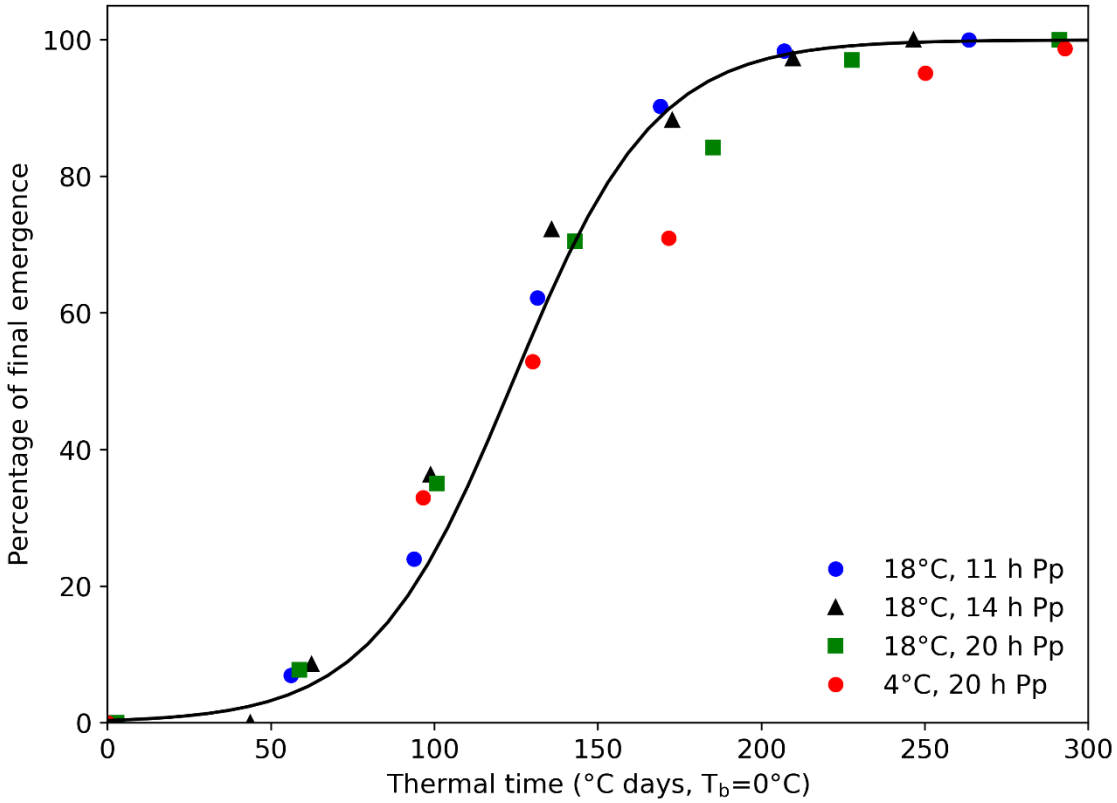


Figure 3.3. Emergence percentage of perennial ryegrass grown under four temperature and photoperiod regimes in controlled environment chambers at Lincoln University, New Zealand. Each data point is the mean of either 13 or 22 individual ecotypes replicated four times. y=100/(1+exp(0.045×(x -123))).

3.3.2 Percentage of plants flowering

Based on the >50% of plants reproductive criteria, 'Kangaroo Valley' was the only ecotype to commit to reproductive development in Experiment 3 (11 h Pp) (Figure 3.4C). Both 'Kangaroo Valley' and 'Medea' were reproductive in Experiment 2 (14 h Pp) (Figure 3.4B) while in Experiment 1 (20 h Pp) (Figure 3.4A) all ecotypes from less than 42° latitude had >70% of plants reproductive. In contrast, those originating from above 42° latitude showed variable results with a trend for lower flowering from mid latitudes. Ecotypes from higher than 48° latitude showed more variability. In Experiment 4, which included a period of cold temperature, all but two ecotypes achieved 50% flowering (Figure 3.4D, Appendix 1).

The percentage of plants reproductive in Experiment 4, 85%, was more than the 67% in Experiment 1, ($P<0.05$, S.E.M = 4.25, $LSD_{0.05}$ = 12.1). The variance ratio of 46.5 for "experiment" was 8 times larger than that of ecotype.

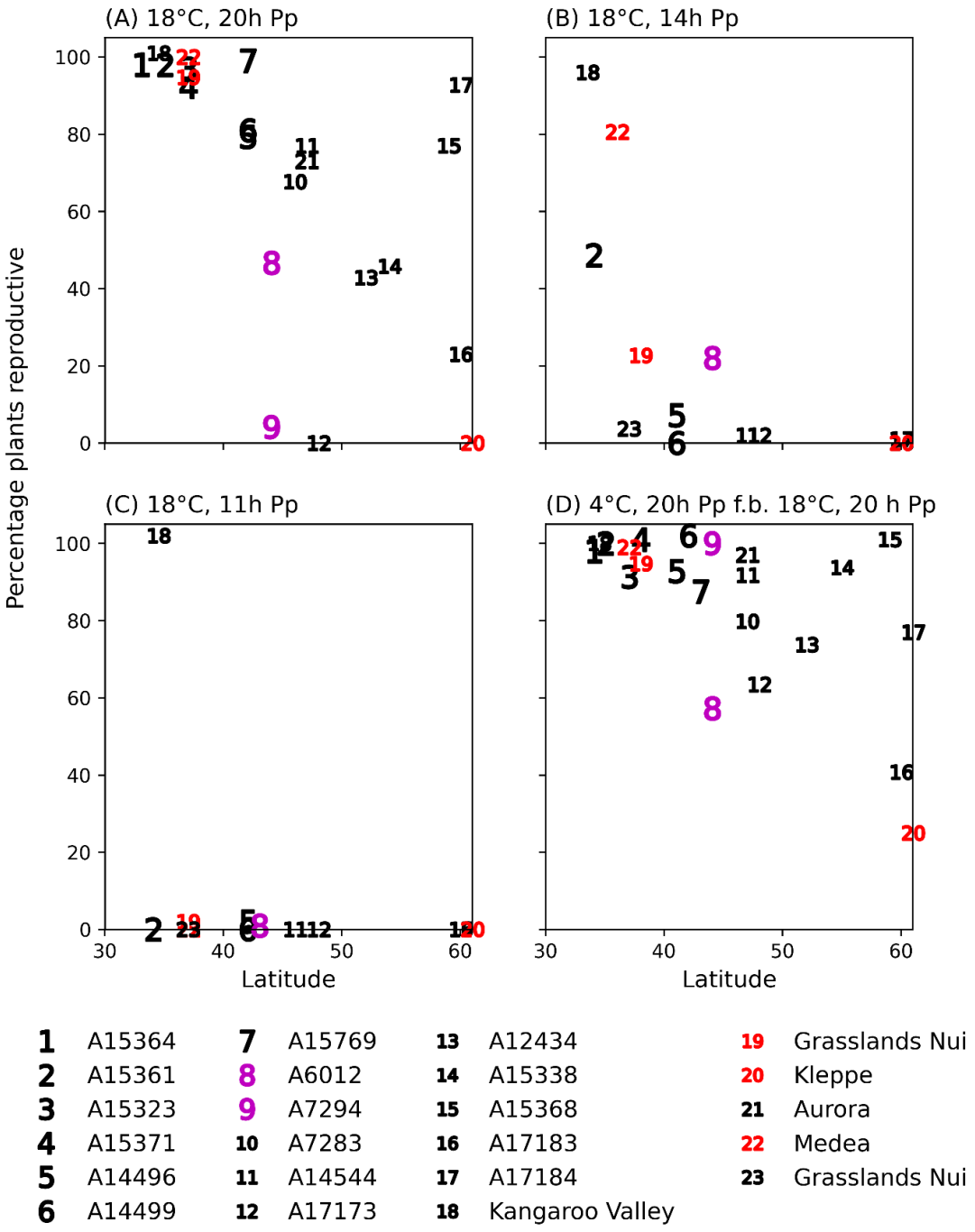


Figure 3.4. Percentage of perennial ryegrass plants that flowered when grown under four temperature and photoperiod (Pp) combinations in controlled environment chambers at Lincoln University, New Zealand. Red = germplasm directly progressed to further study, magenta = germplasm progressed represented by 'Grasslands Impact'. Note, figure nomenclature is temperature and Pp (in hours, h), f.b. is 'followed by'. For example, figure D was a constant temperature of 4°C and constant Pp of 20 h for 12 weeks followed by 18°C and constant Pp of 20 h.

3.3.3 Time to final leaf emergence

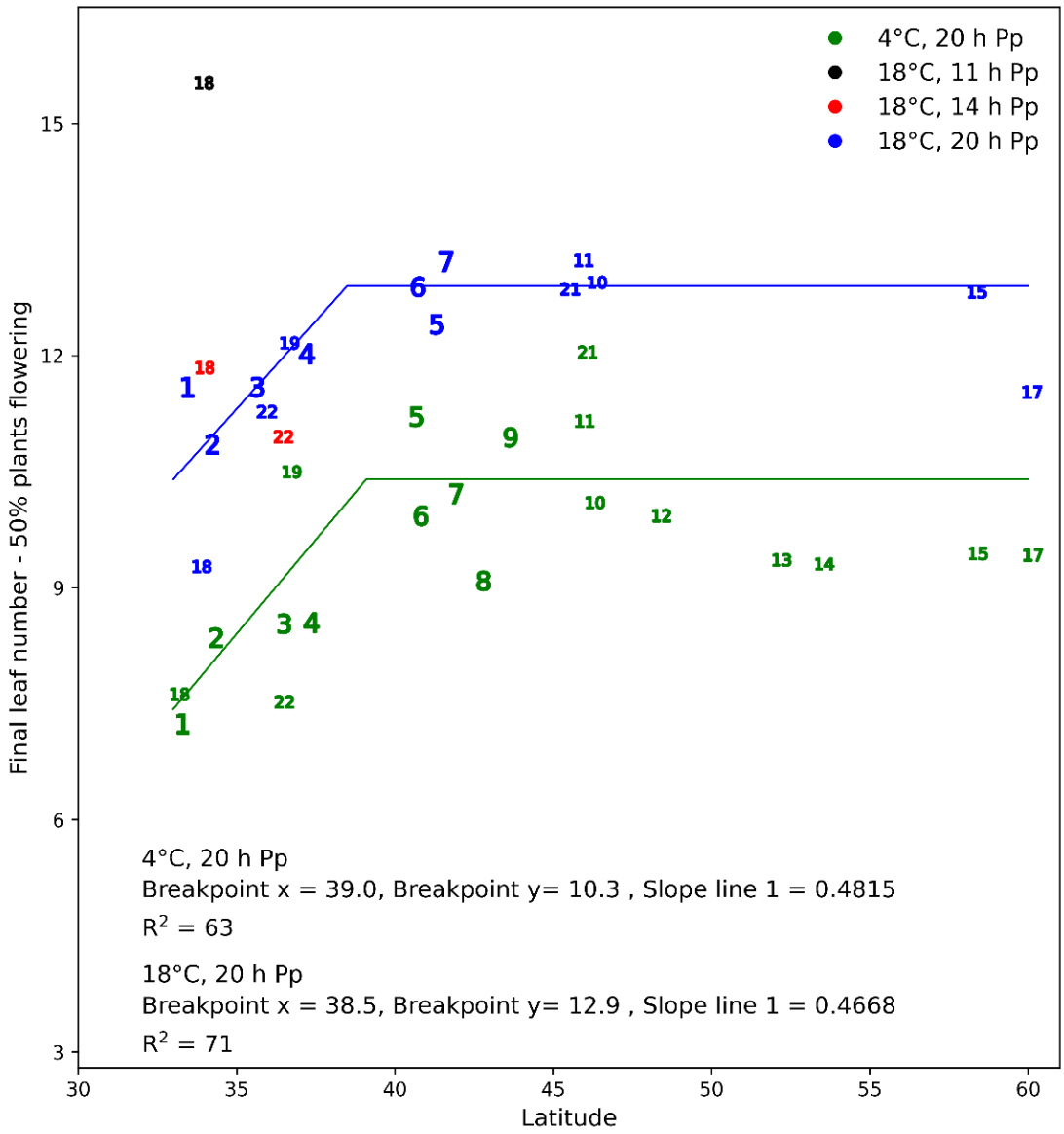
Based on the criteria of 50% of plants flowering, the number of main stem leaves reduced ($P<0.05$) when the latitude of origin was lower than 39° (Table 3.3, Figure 3.5). The minimum number of main stem leaves was ~8 (Table 3.3). For treatments that reached 50% reproductive, the final number of main stem leaves reduced ($P<0.05$) from 12.2 in Experiment 1 to 9.7 in Experiment 4 (S.E.M = 0.149, $LSD_{0.05}$ = 0.45) after vernalisation.

The number of days to final leaf emergence differed with ecotype within individual experiments from 62 days for ecotype #15364 when grown in Experiment 1 to 130 days for 'Aurora' when grown in Experiment 4 (Table 3.4). In thermal time, the duration to final leaf emergence of vernalised plants was ~170°C days shorter than non-vernalised treatments (Table 3.5) a difference of ~2 fewer leaves per stem (Figure 3.5).

Table 3.3. Final number of main stem leaves produced by perennial ryegrass ecotypes, when grown under four temperature and photoperiod combinations in controlled environment chambers at Lincoln University, New Zealand. **Bold** ecotypes reached a minimum of 50% of plants flowering, data includes only reproductive plants. Statistics only include treatments in bold. *Italic* represents non reproductive ecotypes with less than 50% of plants flowering and includes plants that were reproductive.

#	ID/Genotypes	Country of origin	Latitude (°)	Final leaf number			
				4 f.b.18°C[1] 20 hr Pp[2]	18°C 20 hr Pp	18°C 14 hr Pp	18°C 11 hr Pp
1	15364	Morocco	33	**7.1**	**11.6**	-[4]	-
2	A15361	Morocco	34	**8.4**	**10.8**	*15*	*17.4*
3	A15323	Algeria	36	**8.9**	**11.8**	-	-
4	A15371	Tunisia	37	**8.5**	**11.8**	-	-
5	A14496	Turkey	41	**11.0**	**13.1**	*14.2*	*16.6*
6	A14499	Turkey	41	**10.6**	**13.3**	*13.4*	*17.1*
7	A15769	Portugal	42	**10.1**	**13.2**	-	-
8	A6012	Spain	43	**9.7**	*12.9*	*15.3*	*18.1*
9	A7294	Spain	44	**11.0**	*12.9*	-	-
10	A7283	Italy	46	**10.2**	**12.8**	-	-
11	A14544	Italy	46	**10.9**	**13.4**	*15.4*	*19.4*
12	A17173	Ukraine	48	**10.3**	*10.5*	*14.3*	*17.4*
13	A12434	Netherlands	52	**9.7**	*11.4*	-	-
14	A15338	Denmark	54	**9.4**	*12.7*	-	-
15	A15368	Sweden	58	**9.6**	**12.8**	-	-
16	A17183	Norway	59	*14.6*	*11.9*	*13.6*	*14.8*
17	A17184	Norway	60	**9.9**	**12.3**	*12.9*	*15.6*
18	Kangaroo Valley Early		33.5	**7.8**	**9.1**	**12.3**	**16.0**
19	Grasslands Nui		37	**10.7**	**12.9**	*12.3*	*15.4*
20	Kleppe		60	*10.1*	*11.4*	*14.3*	*17.4*
21	Aurora		46	**12.0**	**12.7**	-	-
22	Medea		36	**7.8**	**11.6**	**11.5**	17.2
23	Grasslands Nui[3]		37	-	-	*13.4*	*16.9*
			Mean	9.7 a	12.2 b	11.9	16
			Pvalue	<0.001	<0.001	0.638	-
			S.E.M	0.529	0.579	0.677	-
			$LSD_{0.05}$	1.5	1.65	NS	-

[1]. 12 weeks at 4°C followed by 18°C, [2]. 6 weeks in darkness followed by 20 hour photoperiod, [3]. Fresh seed collected 3 weeks prior to planting, [4]. - not included in individual temperature by Pp treatment. Between 20 hour Pp chamber comparison P = <0.001, S.E.M = 0.149, $LSD_{0.05}$ = 0.451.



1	A15364	**7**	A15769	**13**	A12434	**19**	Grasslands Nui
2	A15361	**8**	A6012	**14**	A15338	**20**	Kleppe
3	A15323	**9**	A7294	**15**	A15368	**21**	Aurora
4	A15371	**10**	A7283	**16**	A17183	**22**	Medea
5	A14496	**11**	A14544	**17**	A17184	**23**	Grasslands Nui
6	A14499	**12**	A17173	**18**	Kangaroo Valley		

Figure 3.5. Final number of main stem leaves produced on perennial ryegrass ecotypes, if 50% of plants reached ear emergence, when grown under four temperature and photoperiod combinations in controlled environment chambers at Lincoln University, New Zealand.

Table 3.4. Days from emergence to final leaf ligule visible (Zadok's growth stage 39) for 23 perennial ryegrass ecotypes/genotypes, when grown under four temperature and photoperiod combinations in controlled environment chambers at Lincoln University, New Zealand

#	ID/Genotype	Country of origin	Latitude (°)	Days to GS39*			
				4 f.b.18°C[1] 20 hr Pp[2]	18°C 20 hr Pp	18°C 14 hr Pp	18°C 11 hr Pp
1	15364	Morocco	33	64	56		
2	A15361	Morocco	34	70	51	-	-
3	A15323	Algeria	36	71	58		
4	A15371	Tunisia	37	69	56		
5	A14496	Turkey	41	77	68	-	-
6	A14499	Turkey	41	82	67	-	-
7	A15769	Portugal	42	75	65		
8	A6012	Spain	43	82	-	-	-
9	A7294	Spain	44	87	-		
10	A7283	Italy	46	81	62		
11	A14544	Italy	46	79	63	-	-
12	A17173	Ukraine	48	82	-	-	-
13	A12434	Netherlands	52	73	-		
14	A15338	Denmark	54	78	-		
15	A15368	Sweden	58	81	65		
16	A17183	Norway	59	-	-	-	-
17	A17184	Norway	60	78	63	-	-
18	Kangaroo Valley		33.5	70	56	68	100
19	Grasslands Nui		37	82	63	-	-
20	Kleppe		60	-	-	-	-
21	Aurora		46	96	63		
22	Medea		36	68	61	87	-
23	Grasslands Nui[3]		37		-	-	
			Mean	77 a	61 b	77	100
			Pvalue	<0.001	0.002	0.035	
			S.E.M	2.78	2.62	3.76	
			$LSD_{0.05}$	7.88	7.49	-	

* GS39 = final leaf ligule visible, [1]. 12 weeks at 4°C followed by 18°C, - treatment included in the temperature by Pp combination but 50% of plants did not achieve GS39.
Experiment comparison P = <0.001, S.E.M = 0.992, $LSD_{0.05}$ = 3.01.

Table 3.5. Thermal time (°C days) from emergence to final leaf ligule visible (Zadok's growth stage 39) for 23 perennial ryegrass ecotypes/genotypes, when grown under four temperature and photoperiod combinations in controlled environment chambers at Lincoln University, New Zealand.

#	ID/ Genotype	Country of origin	Latitude (°)	Thermal time to GS39* (°C days)			
				4 f.b.18°C[1] 20 hr Pp[2]	18°C 20 hr Pp	18°C 14 hr Pp	18°C 11 hr Pp
1	15364	Morocco	33	662	1015		
2	A15361	Morocco	34	796	1005	-	-
3	A15323	Algeria	36	808	1096		
4	A15371	Tunisia	37	762	1082		
5	A14496	Turkey	41	926	1284	-	-
6	A14499	Turkey	41	1011	1216	-	-
7	A15769	Portugal	42	871	1178		
8	A6012	Spain	43	1007	-	-	-
9	A7294	Spain	44	1098	-		
10	A7283	Italy	46	984	948		
11	A14544	Italy	46	951	1211	-	-
12	A17173	Ukraine	48	1009	-	-	-
13	A12434	Netherlands	52	850	-		
14	A15338	Denmark	54	935	-		
15	A15368	Sweden	58	992	1185		
16	A17183	Norway	59	-	-	-	-
17	A17184	Norway	60	935	1163	-	-
18	Kangaroo Valley		33.5	777	1018	1194	1764
19	Grasslands Nui		37	1009	1192	-	-
20	Kleppe		60	-	-	-	-
21	Aurora		46	1251	1211		
22	Medea		36	749	1160	1537	-
23	Grasslands Nui[3]		37			-	-
			Mean	919 a	1131 b	1377	1764
			Pvalue	<0.001	<0.001	0.035	-
			S.E.M	51.2	49.2	93.5	-
			$LSD_{0.05}$	145	141	297	-

* GS39 = final leaf ligule visible for ecotypes where 50% or more of plants were reproductive, [1]. 12 weeks at 4°C followed by 18°C, [2]. 6 weeks in darkness followed by 20 hour photoperiod, [3]. Fresh seed collected 3 weeks prior to planting, - treatment included in the temperature by Pp treatment but 50% of plants did not achieve GS39. Between 20 hour Pp chamber comparison P = <0.001, S.E.M = 20.3, $LSD_{0.05}$ = 61.6.

3.3.4 Phyllochron and spikelets/ear

In Experiment 1, the phyllochron was 106°C days ($P>0.05$) for all ecotypes. In Experiment 4 where ecotypes were vernalised, the phyllochron ranged from 89°C days for 'A15364' to 112°C for 'Kleppe' which did not achieve FI (Table 3.6). The phyllochron was longer for all ecotypes in Experiments 2 and 3 except for 'Kangaroo Valley'. In Experiment 3, the phyllochron slowed after approx. 7 leaves had emerged (Figure 3.6). The longer phyllochron was associated with an increase in leaf length under shorter Pp conditions (Figure 3.7). There were fewer spikelets per ear for ecotypes from lower latitudes (regression slope $P<0.05$) when grown in Experiments 1 and 4 (20 h Pp).

Table 3.6. Mean phyllochron of 23 perennial ryegrass ecotypes/genotypes, when grown under four temperature and photoperiod combinations in controlled environment chambers at Lincoln University, New Zealand.

#	ID/Genotype	Country of origin	Latitude (°)	Phyllochron			
				4 f.b.18°C[1] 20 hr Pp[2]	18°C 20 hr Pp	18°C 14 hr Pp	18°C 11 hr Pp
1	A15364	Morocco	33	89	108		
2	A15361	Morocco	34	92	98	133	175
3	A15323	Algeria	36	91	107		
4	A15371	Tunisia	37	84	108		
5	A14496	Turkey	41	89	106	150	175
6	A14499	Turkey	41	97	106	169	176
7	A15769	Portugal	42	94	105		
8	A6012	Spain	43	99	107	131	145
9	A7294	Spain	44	97	100		
10	A7283	Italy	46	91	105		
11	A14544	Italy	46	90	98	141	147
12	A17173	Ukraine	48	106	118	148	174
13	A12434	Netherlands	52	102	115		
14	A15338	Denmark	54	99	108		
15	A15368	Sweden	58	106	108		
16	A17183	Norway	59	108	120	163	207
17	A17184	Norway	60	106	111	170	182
18	Kangaroo Valley		33.5	96	105	84	107
19	Grasslands Nui		37	103	102	169	196
20	Kleppe		60	112	115	158	161
21	Aurora		46	98	87		
22	Medea		36	90	105	148	168
23	Grasslands Nui[3]		37			162	156
			Mean	98	106	148	167
			Pvalue	0.001	0.079	<0.001	<0.001
			S.E.M	6.35	5.73	6.65	9.42
			$LSD_{0.05}$	12.7	NS	19.1	27

[1]. 12 weeks at 4°C followed by 18°C, [2]. 6 weeks in darkness followed by 20 hour photoperiod, [3]. Fresh seed collected 3 weeks prior to planting.

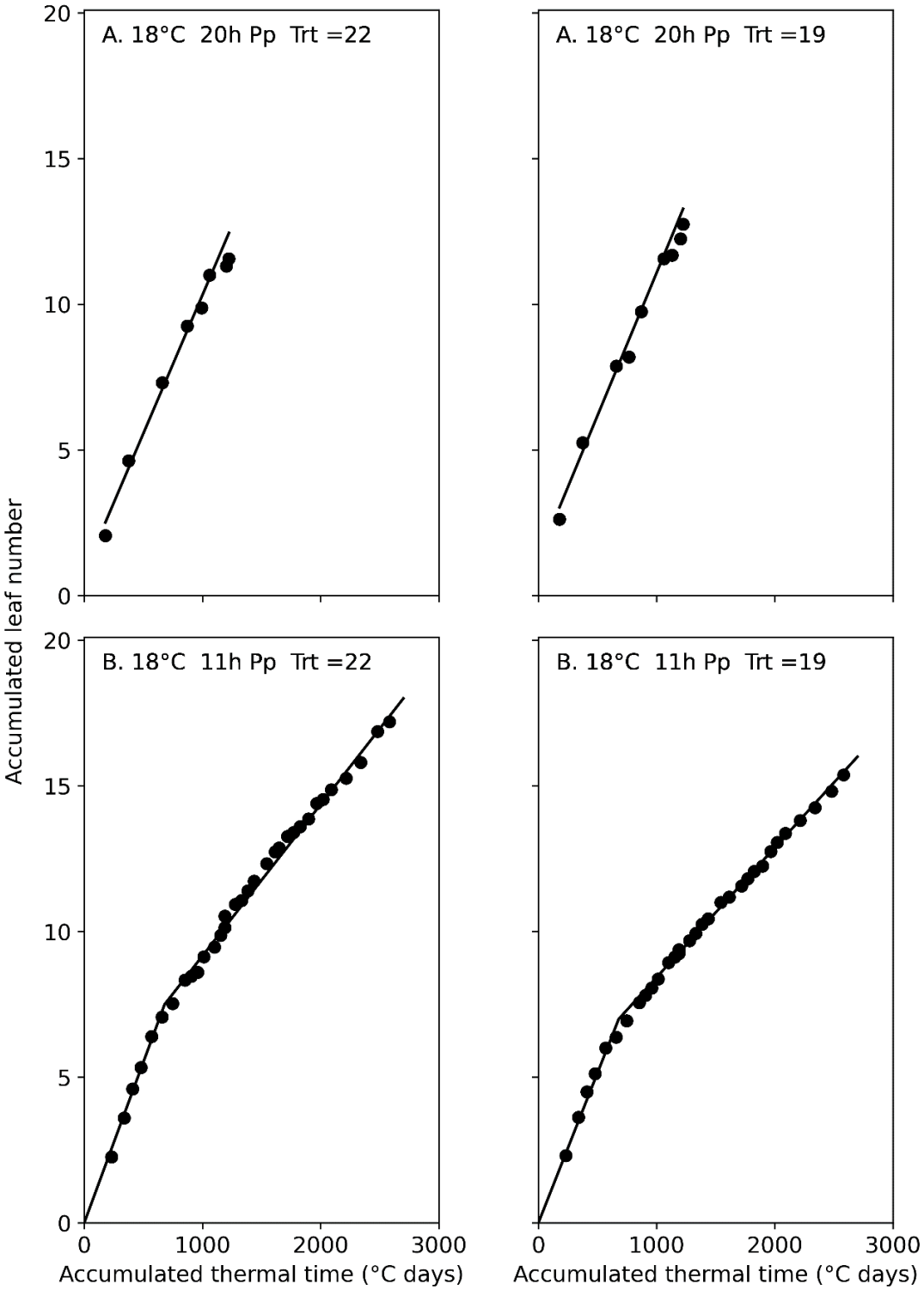


Figure 3.6. Examples from ecotypes 22, 'Medea', and 19, 'Grasslands Nui', perennial ryegrass of a linear leaf appearance relationship with accumulated thermal time when reproductive (A) and how the relationship developed curvature as leaf appearance slowed when leaf length and emergence height increased when grown under constant 18°C and 11 hour Pp, vegetative conditions (B) grown in controlled environment chambers at Lincoln University.

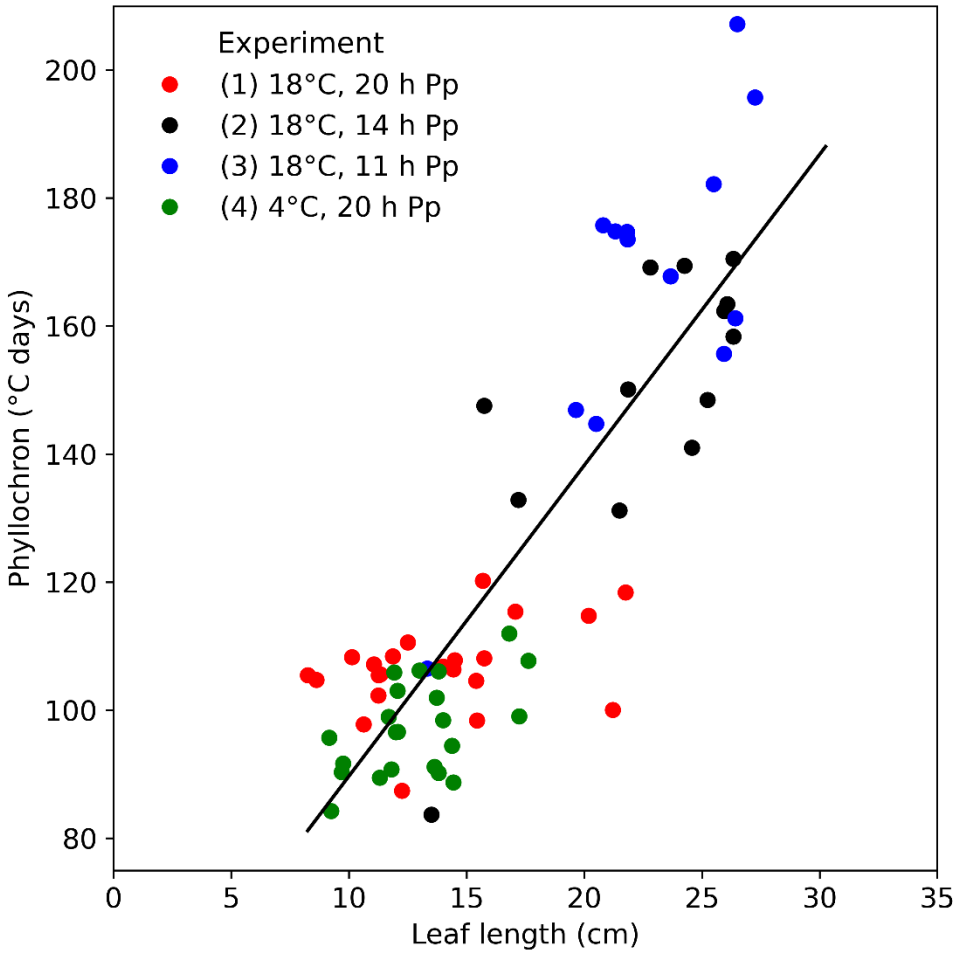


Figure 3.7. Phyllochron and leaf length of the last fully emerged leaf at final assessment of perennial ryegrass ecotypes when grown in four experiments of different temperature and photoperiod combinations in controlled environment chambers at Lincoln University, New Zealand. Line is; Phyllochron = 4.8 (±0.35) × x +41.2 (± 6.14), R^2 =74

Table 3.7. The number of spikelets produced per main stem for 23 perennial ryegrass ecotypes/genotypes, when grown under four temperature and photoperiod combinations in controlled environment chambers at Lincoln University, New Zealand.

#	ID/Genotype	Country of origin	Latitude (°)	Spikelets/ear			
				4 f.b.18°C[1] 20 hr Pp[2]	18°C 20 hr Pp	18°C 14 hr Pp	18°C 11 hr Pp
1	15364	Morocco	33	18.0	17.9		
2	A15361	Morocco	34	18.2	17.0	*18.7*	-
3	A15323	Algeria	36	18.9	17.4		
4	A15371	Tunisia	37	18.0	17.1		
5	A14496	Turkey	41	18.5	20.6	*22.0*	-
6	A14499	Turkey	41	20.4	19.8	-	-
7	A15769	Portugal	42	18.7	20.9		
8	A6012	Spain	43	20.5	21.1	*19.0*	-
9	A7294	Spain	44	20.4			
10	A7283	Italy	46	19.7	20.3		
11	A14544	Italy	46	20.9	19.7	-	-
12	A17173	Ukraine	48	20.5	-	-	-
13	A12434	Netherlands	52	20.1	21.5		
14	A15338	Denmark	54	17.5	22.4		
15	A15368	Sweden	58	21.7	22.8		
16	A17183	Norway	59	*20.4*	23.0	-	-
17	A17184	Norway	60	20.7	20.7	-	-
18	Kangaroo Valley		33.5	20.3	19.6	21.5	21.3
19	Grasslands Nui		37	21.0	22.0	*20.8*	-
20	Kleppe		60	*20.0*	-	-	-
21	Aurora		46	21.0	23.0		
22	Medea		36	17.4	19.6	20.4	-
23	Grasslands Nui[3]		37			-	*23.0*
			Mean	19.5	19.9	20.8	21.3
			Pvalue	0.014	<0.001	0.565	-
			S.E.M	1.236	0.937	1.51	-
			$LSD_{0.05}$	2.48	2.67	NS	-

*****Treatment in red/italic not included in analysis as they did not reach 50% plants flowering. [1]. 12 weeks at 4°C followed by 18°C, [2]. 6 weeks in darkness followed by 20 hour photoperiod, [3]. Fresh seed collected 3 weeks prior to planting.

3.4 Discussion

The objective of this chapter was to identify germplasm with a range of responses to vernalisation, photoperiod and temperature treatments. This was achieved through the selection of genotypes for continued investigation following analysis of results from four experiments where the null hypothesis was rejected.

3.4.1 Flowering

Experiment 1 showed that some perennial ryegrass ecotypes flowered under long days without exposure to vernalising conditions. Under the constant 18°C, 20 h Pp, 15 of the 22 ecotypes went reproductive from latitudes of origin ranging between 33 and 60°N. All genotypes originating from less than 45°N achieved flowering (Table 3.7, Figure 3.4). This result is consistent with Aamlid *et al.* (2000). They showed the cultivar 'Veyo', sourced from central Italy ~42°N, flowered without vernalisation, but all other germplasm, that was selected from latitudes higher than ~50°N, required winter chilling. Cooper (1957) also suggested that up to 20% of plants in some cultivars may flower without the requirement of cold chilling due to the outcrossing nature of *Lolium* species. The 20 h Pp tested is equivalent of mid-summer photoperiod (daylength plus civil twilight) at ~57° of latitude, and is at least three hours longer than those ecotypes collected from less than 45° latitude. When the Pp was reduced to 14 h, 'Medea' and 'Kangaroo Valley' were the only genotypes/ecotypes to flower. The inference is that in shorter photoperiods, either vernalisation or lengthening days are required to induce flowering in most genotypes. These results suggest a genetic mechanism exists for some genotypes to flower in long Pp without exposure to vernalising conditions. Thus, the genotype 'Medea' was progressed for further investigation as it flowered solely in response to photoperiod. In addition, 'Grasslands Nui' was progressed as it flowered strongly in both Experiment 1 and 4 (both 20 h Pp) but did not flower in Experiment 2 (14 h Pp). This shows 'Grasslands Nui' demonstrated a different response for flowering than 'Medea'. 'Grasslands Nui' is also an industry 'standard' genotype against which others are assessed within New Zealand breeding programmes.

'Kleppe' and 'A17183' originating from ~60° latitude were the only ecotypes that did not achieve the 50% of plants flowering criteria in Experiment 4. This indicates these ecotypes

had a strong requirement for further low temperature and/or short days to meet their vernalisation requirement. In the 4°C 20 h Pp chamber, temperatures at the stem apex during the day cycle (lights on) reached ~7°C (Figure 3.1) which is higher than the 6°C reportedly required for vernalisation of perennial ryegrass under long days (Cooper 1960; Evans 1960). However, many other ecotypes did complete vernalisation and were induced to flower in the 20 h Pp. For example, Ecotypes 9, and 14 reached more than 90% of plants flowering compared with less than 40% under 18°C conditions. Equally Aamlid *et al.* (2000) showed that genotypes from lower latitudes underwent reproductive development when vernalised in long days, while those from more northern latitudes were variable in their response. Thus, genotype differences in response to photoperiod requirements during the primary induction phase may be important in determining time of flowering, particularly for spring sowing dates in lengthening photoperiods. Therefore, 'Kleppe' which showed limited flowering in all conditions was progressed to represent genotypes that appear to have an obligate requirement for short Pp and/or cool temperatures to achieve vernalisation.

Ecotypes 'A6013' and 'A7294' are also of interest because they demonstrated a requirement for vernalisation when they flowered in Experiment 4, which separates them from 'Kleppe'. Additionally, they did not flower in Experiments 2 or 3 (Figure 3.4). 'A6013' and 'A7294' could not be progressed as there was insufficient seed supply. Both ecotypes were collected from northwest Spain, the same area as the populations that were included in the background breading of 'Grasslands Impact'. Thus, 'Grasslands Impact' was chosen as a substitute for this response and included for further study. Germplasm from north west Spain has become important in New Zealand breeding programmes since 1986 and subsequent genotypes are typically later flowering than 'Grasslands Nui' (Stewart 2006).

3.4.2 Phenophase durations

The time to emergence was similar for all experiments when soil temperature was used to calculate thermal time (T_b= 0°C) at 123°C days (Figure 3.2). This is similar to the 133°C days for field emergence reported by Moot *et al.* (2000) for 'Grasslands Nui'. Since soil temperature during germination was often different from the air temperature, due to

radiant heat from the chamber lighting (Figure 3.1). When expressed in days, emergence was slower at 4°C than 18°C. Thus, the phenophase from sowing to emergence should be quantified using soil temperature to provide an accurate estimation of the temperatures experience by the seedling during emergence (Jamieson *et al.* 1995b).

For those ecotypes that reached final leaf emergence in Experiments 1 and 4, the duration from emergence to final leaf ligule was longer when not vernalised in all three assessment criteria; days (Table 3.4), thermal time (Table 3.5) and the number of main stem leaves (Table 3.3, Figure 3.5). The mean number of main stem leaves following vernalisation in Experiment 4 was 9.6, or ~2.5 fewer than the 12.2 when grown in Experiment 1. The minimum number of main stem leaves was 7.8 produced by 'Medea' (ecotype 'A15364' showed non perennial ryegrass attributes) which suggests this is the lowest number that could emerge before flowering. This is similar to the 7.5 leaves identified as the minimum final leaf number of wheat (Brooking *et al.* 1995; Jamieson *et al.* 1998b). These data suggest that induction to flower was rapid following emergence into 20 h Pp when grown in cool (4°C) conditions.

The Tt from emergence to GS39 was 234°C days shorter in Experiment 4 (913°C days) compared with Experiment 1, constant 18°C, 20 h Pp (1148°C days) (Table 3.5). Of plants that flowered in both treatments, those grown in Experiment 1, produced an extra 2.5 leaves at an equivalent phyllochron of 97°C days, similar to the mean phyllochron for chambers that included reproductive plants. Thus, the increased duration to GS39 was explained by the production of these extra leaves when plants were not exposed to vernalising temperatures.

3.4.3 Phyllochron

The mean phyllochron increased from ~100°C days for ecotypes which were reproductive in 20 h Pp chambers, to ~170°C days in 18°C temperatures with 11 h Pp where all ecotypes were vegetative, except for 'Kangaroo Valley'. These values are consistent with the range of phyllochron suggested by Black *et al.* (2002) at 101°C days and 167°C days reported by Romera *et al.* (2009). The increase in phyllochron was associated with an increase in individual leaf length measured at the final assessment (Figure 3.7). Skinner and Nelson

(1994) showed a similar decline in phyllochron for controlled environment chamber grown tall fescue as the duration required for each subsequent leaf to emerge through the previous leaf sheath increased. If the stem apex was raised with each leaf, as occurs during stem extension, an increase in phyllochron due to growth is not required and the relationship is linear (Figure 3.6A). However, the relationship showed curvature and a slowing in phyllochron at ~HS7 (Figure 3.6B). This pattern of leaf appearance has also been reported for wheat by Jamieson *et al.* (1995b) and Miglietta (1991) who suggested phyllochron slows with Haun stage. The implication of this is discussed further in Sections 4.3.3 and 4.4.6.

3.5 Conclusions

These results have identified the flowering responses in a diverse range of ecotypes with the objective to identify four to progress into detailed experimentation. The response types identified included;

- Group 1. Non obligate for vernalisation, obligate for long Pp. Achieve flowering in response to 20 h Pp only, thus vernalisation was not obligate e.g. 'Grasslands Nui'.
- Group 2. Non obligate for vernalisation, obligate for medium Pp. Flowering in response to Pp only where Pp longer than 11 h induced FI, thus vernalisation was not required for flowering, e.g. 'Medea'.
- Group 3. Obligate vernalisation, satisfied under long Pp e.g. ecotypes originating from latitudes of ~45-60°N, substituted with 'Grasslands Impact' for further experimentation.
- Group 4. Obligate vernalisation. Ecotypes that did not flower in any experiments, thus demonstrating a strong requirement for short days, or extended duration of cold, e.g. 'Kleppe'.

4 VERNALISATION EXPERIMENT

Objective 2

- To quantify the vernalisation and thermal time requirements for the phenophases up to final leaf emergence of four representative perennial ryegrass genotypes under long and short photoperiods.

Null hypothesis:

- All genotypes will have the same temperature and photoperiod response to induce reproductive development.

4.1 Introduction

This chapter describes the temperature and photoperiod interactions during the primary induction phase of four contrasting perennial ryegrass genotypes and therefore relates to Objective 2. The obtainment of FI following exposure to cool temperatures is the main mechanism used to determine if flowering occurs within a perennial ryegrass genotype. If genotype specific vernalisation requirements are not met, flowering does not occur, except in a limited number of genotypes where long Pp may induce flowering (Cooper 1960; Aamlid *et al.* 2000). Additionally, progress towards saturation of the vernalisation requirement shows a facultative response whereby increased exposure reduces the time to flowering (Evans 1960; Langer 1979).

This research utilises the genotypes 'Medea', 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact', identified in Chapter 3, to explore the influence of temperatures between 4 and 18°C and Pp on progress toward FI. Exposure to vernalising conditions is considered in two forms; 1) the duration required for 50% of plants to flower (referred to as V_{base}) and, 2) the duration required to reach saturation of the response (V_{sat}), defined as the fewest number of leaves at final leaf emergence. Both V_{base} and V_{sat} will be determined retrospectively. Thus, this chapter describes the response of the four genotypes to eight individual temperature by Pp combinations.

To do this, each plant was subjected to three phases of environmental conditions, 1). pre-treatment, 2). treatment and 3). post treatment conditions. The pre-treatment and post treatment conditions were the same at constant 18°C, 17 h Pp. Pre-treatment lasted from sowing to HS1.5 (Haun 1973), or approx. three weeks (Figure 4.1). During the treatments

phase, a range of primary induction conditions were applied in two-week intervals lasting from 0 – 12 weeks. The post treatment conditions of constant 18°C, 17 h Pp, enabled plants to express the impact of the environmental conditions applied during the treatment phase. The use of HS removes the confounding effects of temperature *per se* on development during vernalisation, when plants will develop a different number of leaves based on the duration of the treatment phase. This removes the measurement of "days" which is confounded because plants develop (initiate leaves) at different intervals dependent on ambient temperature.

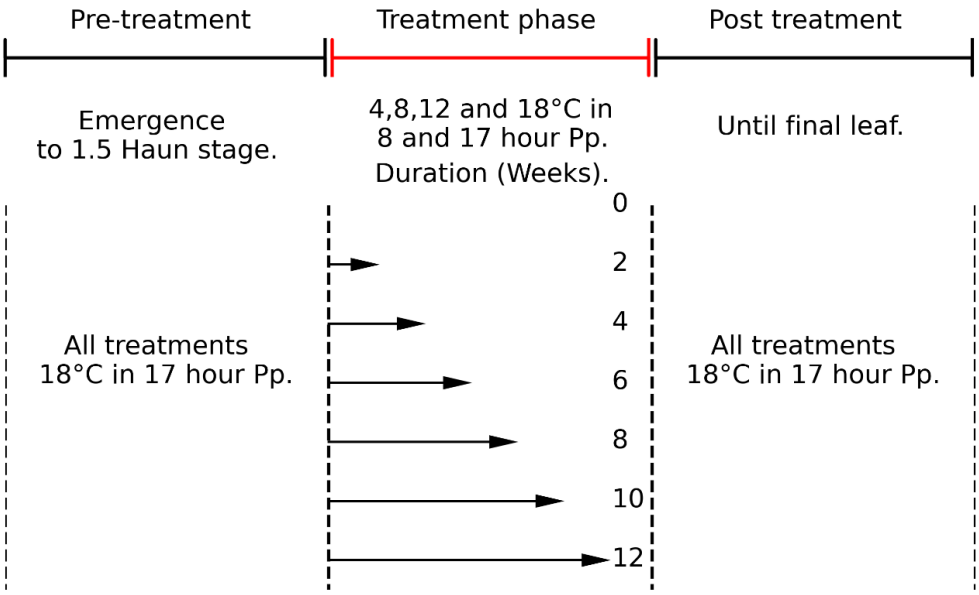


Figure 4.1. Treatment structure to investigate the influence of environmental conditions during primary induction on flowering time of four perennial ryegrass genotypes.

First, the temperature exposure requirements to induce flowering on 50% of main stems was investigated. This is used to define the shortest exposure requirement (V_{base}) for flowering in which the duration to final leaf appearance is the longest. The interval reduces to a minimum when the number of leaves produced is minimised, which corresponds to the developmental stage of V_{sat}. Primordium production is expected to follow a trilinear relationship where FI and TS are represented by breakpoints of the relationship (Figure 4.2) (Section 2.4). FI is calculated retrospectively based on the time when the rate of primordium production increases on the stem apex. Double ridge, an

observable developmental stage, occurs sometime after FI, prior to TS, and is not included in the analysis. Obtainment of TS means labile primordium, that could produce a leaf or a spikelet, are committed to either outcome where the appearance of remaining leaves determines the duration from TS to final leaf appearance. All treatments will be plotted (vegetative and reproductive) relative to HS. This allows calculation of the duration between the FI, TS and final leaf stages as influenced during the primary induction phase (Chapter 2). Finally, the results are used to develop equations to estimate the time to V_{base} and V_{sat} which could be used for modelling purposes (Objective 2).

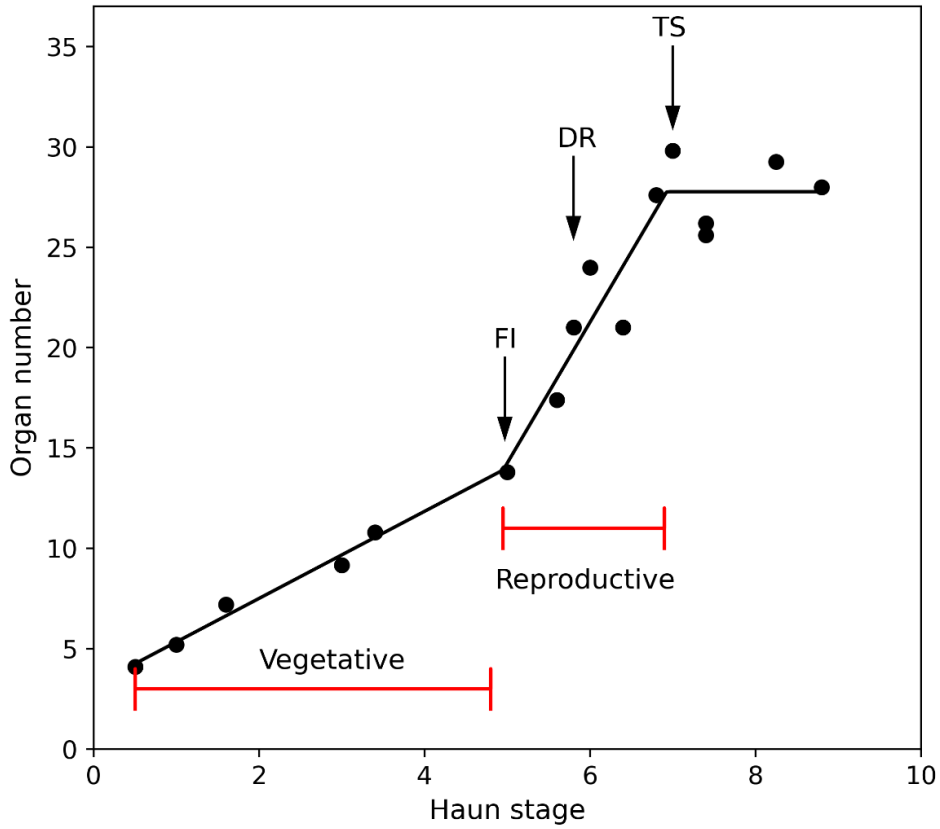


Figure 4.2 Example of the expected three straight line model used to describe the number of organs in reproductive plants relative to Haun Stage (number of main stem leaves produced), genotype 'Medea', 12°C, 8h Pp for 2 weeks followed by 18°C 17 h Pp.

4.2 Materials and methods

4.2.1 Sowing and establishment

Seeds were sown directly on to damp potting mix housed in 22 cm deep, by 4 cell root trainers each containing approximately 3 L of 9 month potting mix. Two seeds were sown per cell to ensure each cell contained at least one plant. To control temperature at seed and subsequent apex level, seeds were covered with 2 cm of wet 'Grade 3' vermiculite, leaving a 1 cm 'air' space to the top of the root trainer. Root trainers were covered with commercial grade tinfoil with a 5 mm hole above the sown seed to allow emergence. The tinfoil reflected radiant heat generated by the chamber lighting and thus the combination of vermiculite and tinfoil helped maintain the target temperature at stem apex level. Root trainers were placed in large plastic trays to facilitate bottom watering. Phase 1 involved germination and emergence to Haun stage 1.5 (Haun 1973) of all seeds at constant 18°C, 17 h photoperiod. At Haun 1.5, seedlings were thinned to one plant per cell and they were transferred into their respective treatment conditions. Following exposure to treatment conditions, plants were transferred to the post treatment conditions (Figure 4.1).

Prior to the production of a full leaf canopy, pots were top watered to ensure adequate moisture for germination and early growth, and that the vermiculite remained wet for insulation purposes. Following full canopy closure, pots were bottom watered only. Pots were watered using standard tap water that was acclimatised in 20 L containers inside each controlled environment chamber for at least 24 hours, with exception of the control chamber that used tap water. For each additional two leaves produced, pots were fertilised using "Yates Thrive" all-purpose soluble fertiliser at 16 g per 9 L of water (undiluted nutrient content (%) N 25, P 5, K 8.8, S 4.6, Mg 0.5, Fe 0.18, B 0.005, Cu 0.005, Zn 0.004, Mo 0.001). Thus, treatments at 18°C were fertilised more frequently than those under cooler conditions.

4.2.2 Experimental treatments

Eight sets of environmental conditions investigated four temperature regimes, 4, 8.1, 12 and 18°C in combination with two Pp, 8 and 17 h (Table 4.1). Within each temperature treatment, plants were exposed for 0 (control), 2, 4, 6, 8, 10 or 12 weeks giving 50 individual treatment combinations.

Air temperature was measured inside a radiation shield at two locations within each chamber hourly. In addition, soil temperature was measured at seed depth (3 cm), at three locations inside each controlled environment chamber using Hobo® U12 Outdoor/Industrial data loggers equipped with four TMCx-HD temperature sensors. Achieved apex temperatures were close to target temperatures for the majority of the experiment (Figure 4.3).

Table 4.1. Temperature and photoperiod combinations used in controlled environment chambers to investigate the vernalisation response for flowering of four perennial ryegrass ecotypes at Lincoln University, New Zealand.

Experiment #	Treatment phase		Post treatment phase	
	Temperature (°C)	Photoperiod (hours)	Temperature (°C)	Photoperiod (hours)
5	4	8	18	17
6	4	17	18	17
7	8.1	8	18	17
8	8.1	17	18	17
9	12	8	18	17
10	12	17	18	17
11	18	8	18	17
12 (Control)	18	17	18	17

Plants remained in post treatment conditions until either the final leaf on the main stem had emerged or plants that remained vegetative had produced ~20 main stem leaves with no sign of reproductive development on any dissected stem apices.

4.2.3 Chamber conditions

All experiments were conducted in the same Conviron controlled environment chambers described in Section 3. The actual conditions obtained are reported in Figure 4.3.

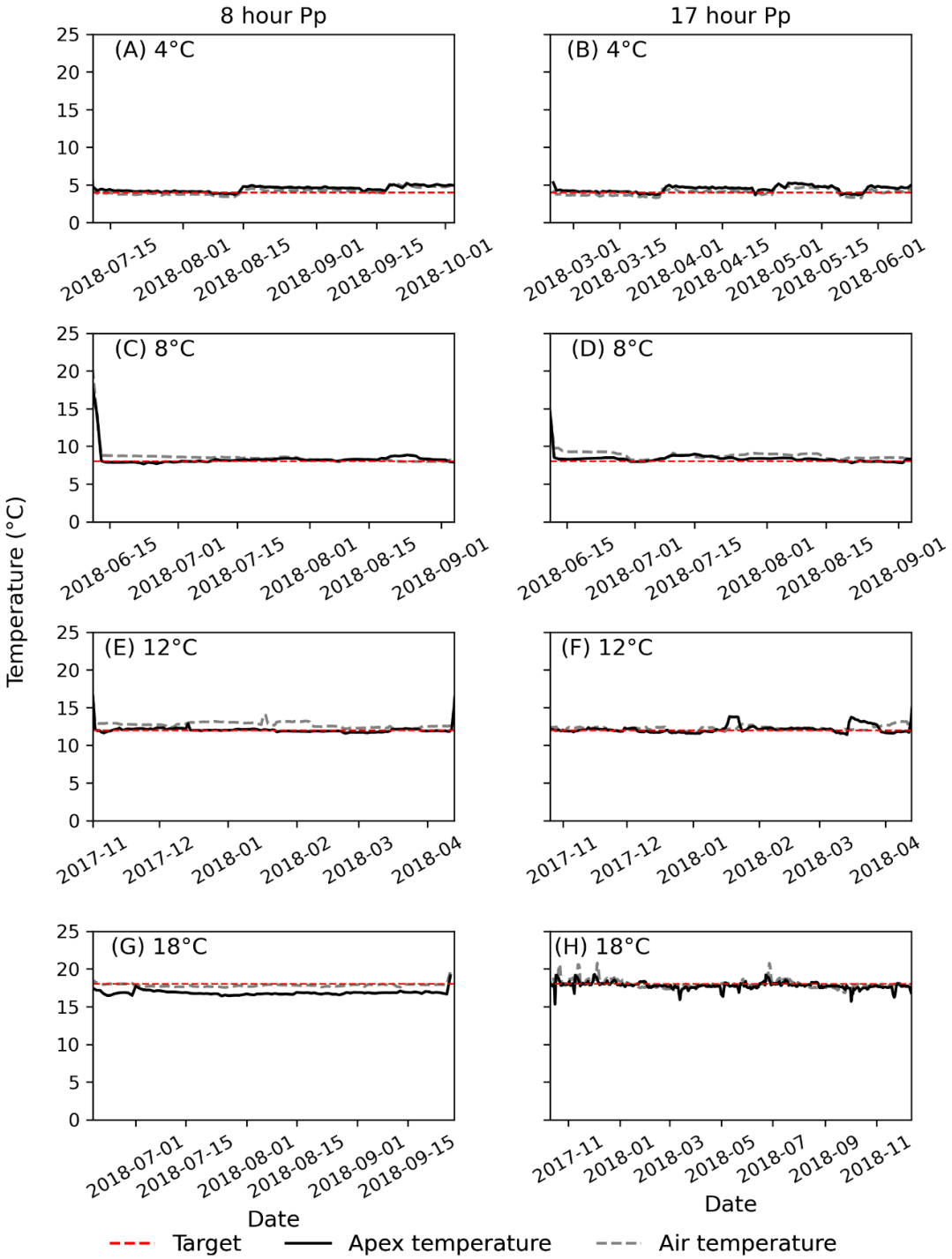


Figure 4.3. Air, apex (3 cm depth) and target temperature for eight controlled environment chambers used for primary and secondary induction of perennial ryegrass at Lincoln University, New Zealand.

4.2.4 Measurements

At HS2, the main stem of each plant was tagged using coloured electrical wire. Tags were placed underneath the latest leaf to emerge to ensure they could not be dislodged. Tags were moved upwards on the main stem at approximately 2-3 leaf intervals to track the main stem and leaf emergence. At approximately weekly intervals, 3-5 plants were extracted from each chamber and the leaves removed to expose the stem apex. For each plant the Zadok's stage, number of emerged leaves, length of last emerged leaf and emerging leaf, number of leaves yet to emerge and the number of primordium were counted. Observations of apex dome elongation, double ridge and terminal spikelet were recorded as described in Section 2.4.1. Following final leaf emergence on at least 50% of plants within a treatment, the remaining plants were grown to full head emergence, when the number of main stem leaves produced and spikelet number were counted on all remaining plants.

4.2.5 Statistical analysis

Treatments were considered reproductive if more than 50% of plants at the final assessment were reproductive i.e. had either produced a flag leave ligule or achieved double ridge stage when dissected. Generally, treatments had 9 or more plants at the final assessment but occasionally only 3-5 remained, usually in treatments that were not reproductive after 12 months, where regular dissections had reduced the number of plants available.

Data were collated in MS excel and interrogated utilising the packages inside the Python™ programming language. Linear regression used the "olm" (Ordinary Least Squares) method in the "statsmodels" package of Python (Seabold & Perktold 2010). Trilinear models (three straight lines) were fitted to organ number by Haun stage for data where treatments achieved more than 50% of plants flowering using the 'curve_fit' method inside the 'scipy' package of Python. The model was defined as Equation 21.

Equation: For:

$$\begin{array}{cc} M = M & x \geq x2 \\ M - b2 \times (x2 - x) & x1 \leq x > x2 \\ M - b2 \times (x2 - x1) - b1 \times (x1 - x) & x < x1 \end{array}$$ Equation 21

Where:

M	is the maximum organ number
$x1$	is break point 1 on the x axis (the time of FI)
$x2$	is break point 2 on the x axis (the time of TS)
$b1$	is the slope of line 1
$b2$	is the slope of line 2

Starting values for line fitting provided were, $M = \max y\ axis$ value, $x1 = x2 - 2$, $x2 = \max x - 2.5$, $b1 = 1.75$, $b2 = 10$.

*Note: if optimal line fitting was not achieved, starting values were adjusted on individual treatments to achieve optimal fit.

For treatments that did not achieve 50% of plants flowering, data of organ number and Haun Stage were described using two straight lines fitted using the 'curve_fit' method (Equation 22).

Equation: For:

$$\begin{array}{cc} b1 \times x + y0 - b1 \times x0 & x < x0 \\ b2 \times x + y0 - b2 \times x0 & x > x0 \end{array}$$ Equation 22

Where:

$x0$	is break point on the x axis
$y0$	is break point on the y axis
$b1$	is the slope of line 1
$b2$	is the slope of line 2

Starting values for line fitting provided were, $x0 = 0.25 \times \max x$ value
$y0 = (0.25 \times \max x \text{ value}) \times 2$, $b1 = 2$, $b2 = 1$.

Where analysis involved rate of development, the treatment of weeks was converted to days and the rate of development/progress per day calculated as the inverse of time for the saturating duration at each temperature.

4.3 Results

4.3.1 Apex state on transfer from treatment conditions

For ‘Medea’, FI was not observed before transfer when the treatments occurred under 8 h Pp, regardless of temperature (Figure 4.4A,C,E,G). Under a 17 h Pp, ‘Medea’ reached FI prior to 12 weeks at all temperatures and at 12°C had obtained TS on transfer after 10 weeks (Figure 4.4F).

‘Kleppe’ and ‘Grasslands Nui’ showed no visual sign of FI at transfer in any of the treatment combinations tested (Figure 4.4D).

For ‘Grasslands Impact’ no FI occurred under an 8 h Pp, regardless of temperature (Figure 4.4A,C,E,G). In 17 h Pp, changes on the stem apex were observed at 8°C after 8 weeks (Figure 4.4D) which provided the shortest duration required for FI. At 4°C, ‘Grasslands Impact’ required 12 weeks before an indication of FI was visible (Figure 4.4B).

4.3.2 Haun stage on transfer

The Haun stage on transfer increased for all genotypes as the temperature and duration of exposure increased during the treatment phase (Figure 4.5). There was no difference between 8 and 17 h Pp.

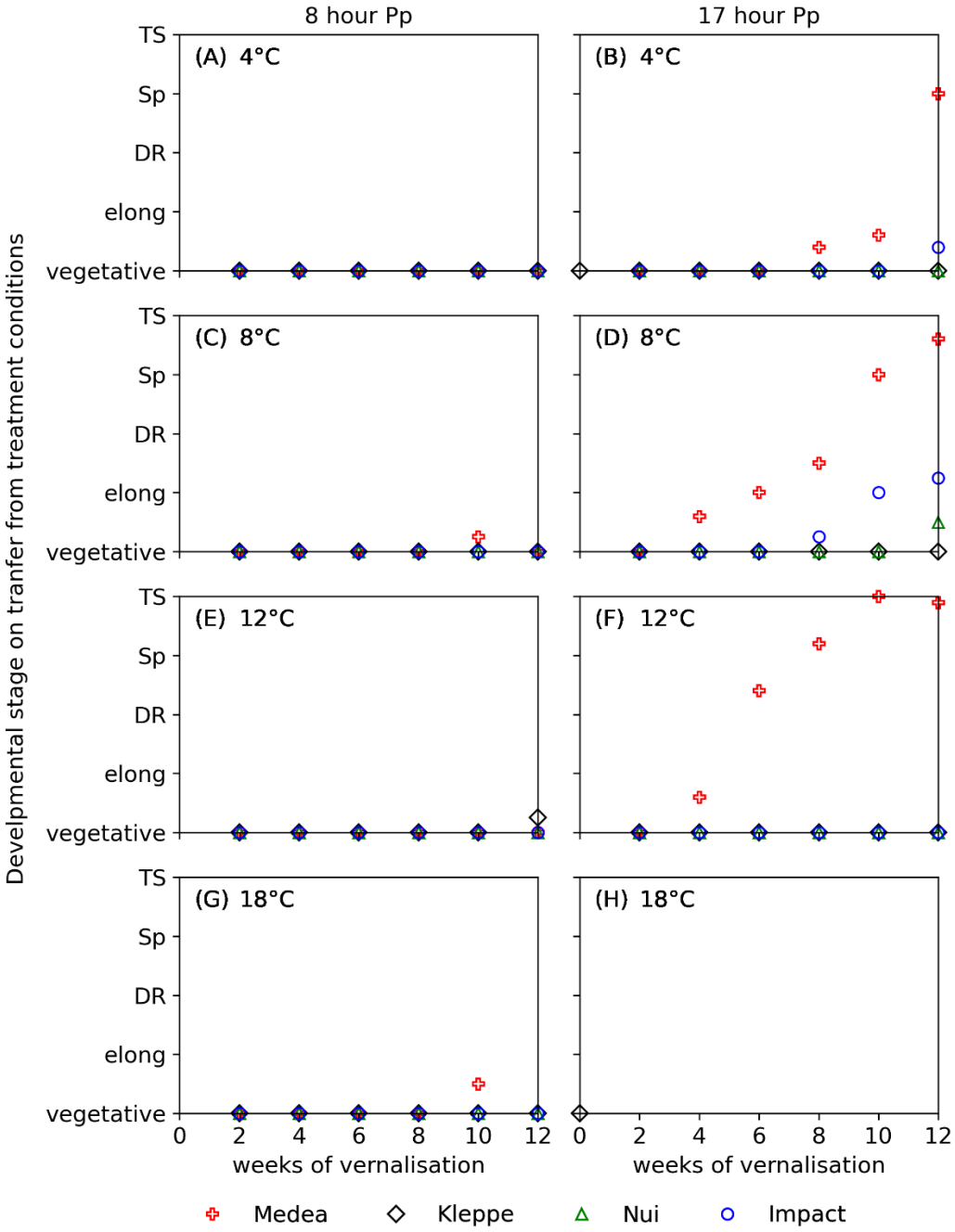


Figure 4.4. State of apex development on the main stem of four perennial ryegrass genotypes on the day of transfer from treatment conditions in to constant 18°C, 17 hour Pp. All treatments grown in controlled environment chambers at Lincoln University, New Zealand. elong = apex elongation, DR = double ridge, Sp = spikelet initiation, TS = terminal spikelet formation.

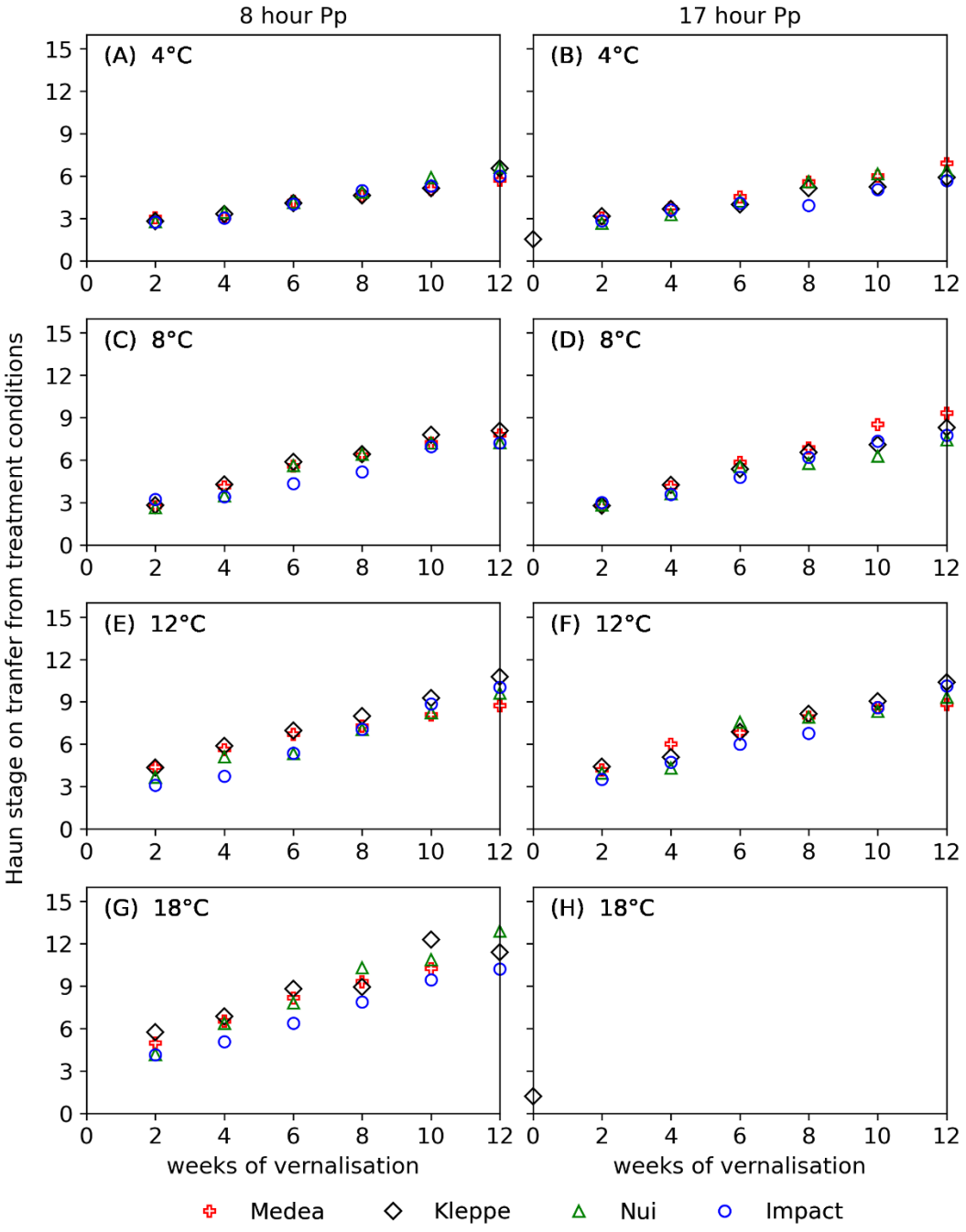


Figure 4.5. Haun stage of four perennial ryegrass genotypes on transfer from treatment conditions in to constant 18°C, 17 hour Pp following exposure to four temperatures at two photoperiods (Pp) for up to 12 weeks. All treatments grown in controlled environment chambers at Lincoln University, New Zealand.

4.3.3 Phyllochron

Haun stage followed a conservative pattern among genotypes when described using thermal time, above T_b = 0°C (Figure 4.6). Phyllochron described by a split line regression with a single break at ~HS8, assuming 125°C days from sowing to emergence (Chapter 3). With this model, phyllochron was initially 105°C days until HS7.8 (946°C days ±25) after which it increased to 188°C days.

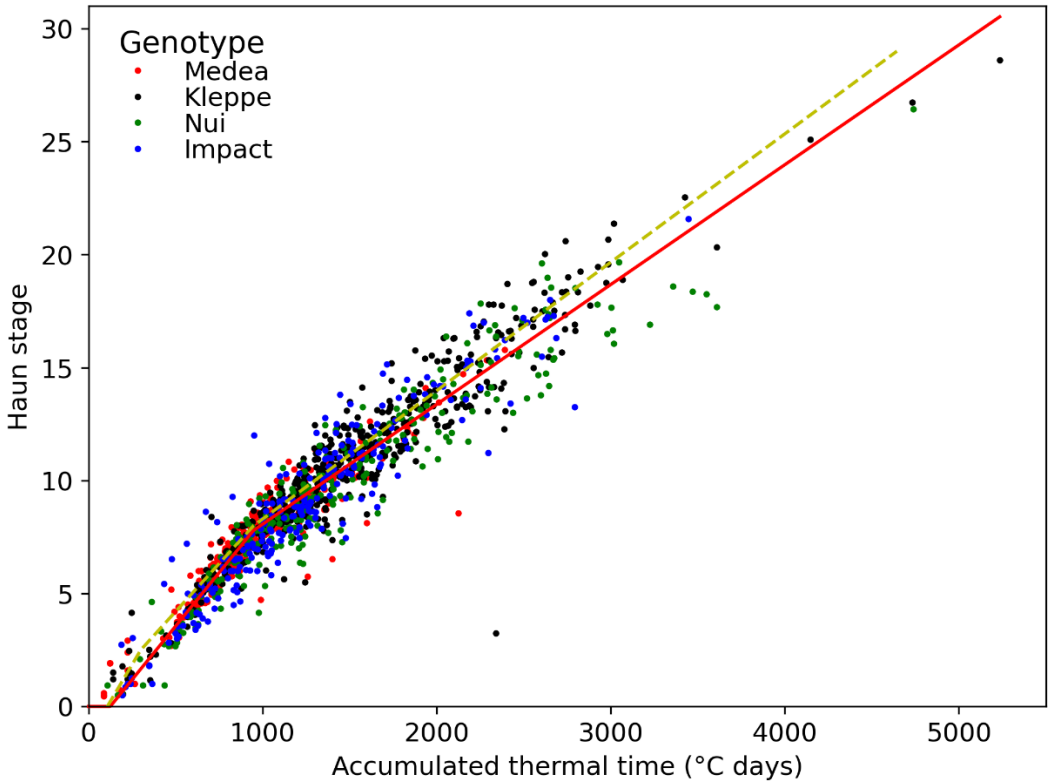


Figure 4.6. Haun stage against thermal time above a base temperature of 0°C for four perennial ryegrass genotypes grown at four temperatures, in two photoperiods in controlled environment chambers at Lincoln University. Line = 0 to emergence at 125°C days, Breakpoint $\boldsymbol{y}$ = 7.78, breakpoint $\boldsymbol{x}$ = 947, slope line 1 = 0.0095, slope line 2 = 0.0053, R^2 = 0.91. Yellow line is a similar model for wheat for comparison from Brown *et al.* (2013).

4.3.4 Percentage plants flowering

For clarity, all treatments discussed below have been exposed to treatment conditions as indicated and results include time spent in post treatment conditions of constant 18°C, 17 h Pp. For example, if treatment was 2 weeks at 4°C, this was followed by a constant 18°C, 17 h Pp until either final leaf emergence or ~20 main stem leaves emerged.

'Medea' produced ears following all treatments (Figure 4.7A and B), including the control which was held at a constant 18°C (Figure 4.7B). No other germplasm showed significant reproductive development when held at 18°C. This suggests an obligate vernalisation requirement for all other genotypes.

'Kleppe' required at least six weeks of exposure to temperatures between 4 and 12°C under 8 h Pp (Figure 4.7C) for flowering to occur. However, in a 17 h Pp, 'Kleppe' required 10 weeks at 4°C to achieve 50% of plants reproductive (Figure 4.7D).

Under 8 h Pp, the response of 'Grasslands Nui' was similar to 'Kleppe'. That was at least six weeks of exposure between 4 and 12°C was required for flowering to occur (Figure 4.7E). However, in a 17 h Pp, 'Grasslands Nui' was vernalised by temperatures at or below 8°C, but required approximately two weeks longer than the corresponding 8 h Pp treatments (Figure 4.7E). Additionally, 'Grasslands Nui' had a low number of plants that reached head emergence in a range of treatments.

'Grasslands Impact' was intermediate of 'Medea' and 'Grasslands Nui'. In most cases 'Grasslands Impact' required two fewer weeks of treatment exposure than 'Grasslands Nui' when temperatures were at or below 12°C, in both 8 and 17 h Pp (Figure 4.7G and H). The shortest primary induction duration required for 'Grasslands Impact' to achieve 50% flowering was at 8°C, in 8 h Pp (Figure 4.7G).

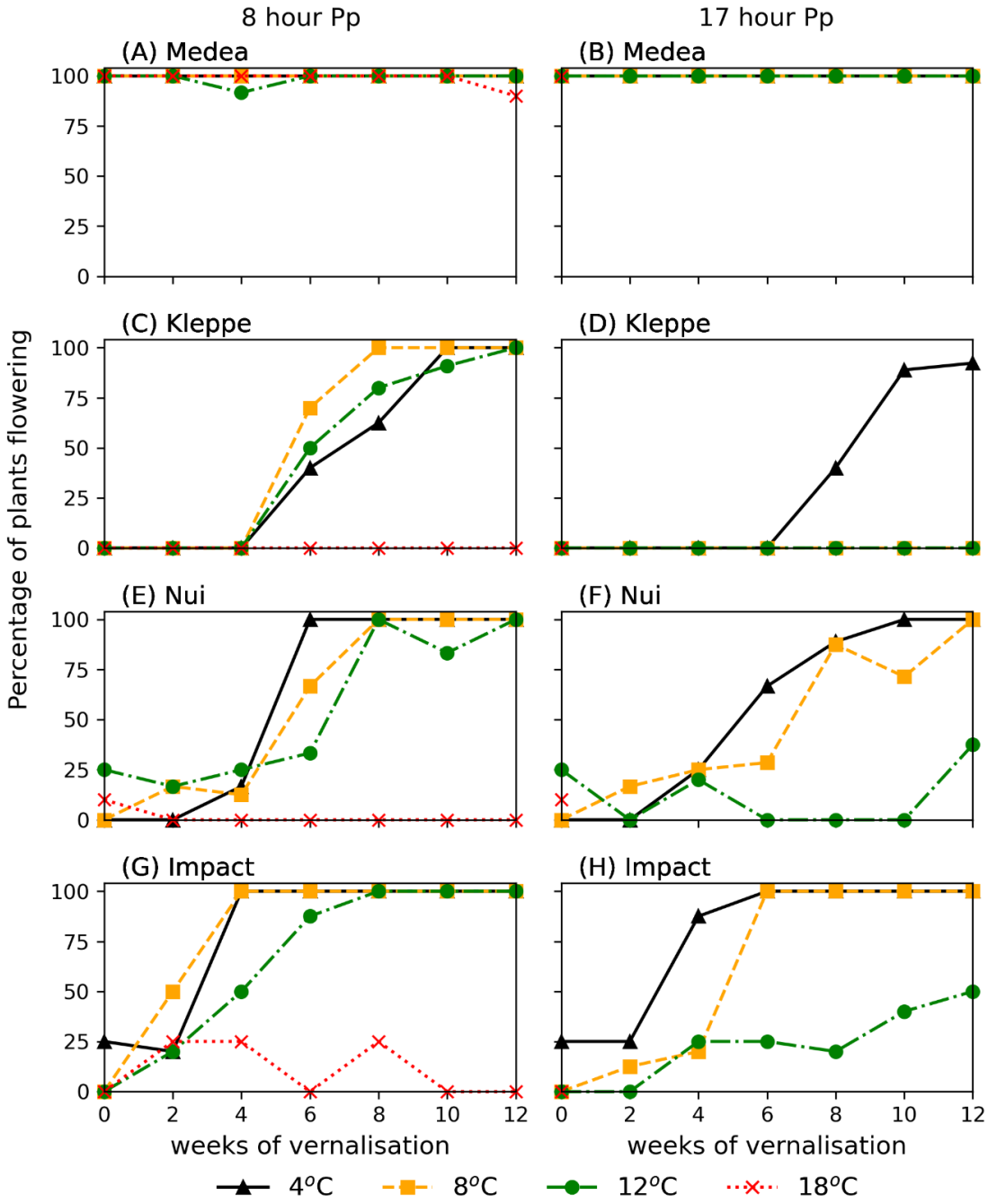


Figure 4.7. Percentage plants flowering of four perennial ryegrass genotypes following different durations of vernalisation in either 8 or 17 hour photoperiod (Pp), before transfer to a constant 18°C, 17 hour Pp when grown in controlled environment chambers at Lincoln University, New Zealand.

4.3.5 Duration to 50% flowering

The shortest duration of exposure to treatment conditions where 50% of plants flower is V_{base}. For example, in 'Medea' all plants flowered in the post treatment conditions, without requiring exposure to vernalising conditions, therefore, 'Medea' has a V_{base} of 0 days (Table 4.2). Alternatively, 'Kleppe' and 'Grasslands Nui' required a minimum of 42 days exposure at either 8 or 4°C respectively when exposed to 8 h Pp conditions.

Table 4.2. Minimum number of weeks (days) of primary induction required to achieve 50% of plants flowering (V_{base}) for four genotypes of perennial ryegrass grown at four temperatures in either 8 or 17 hour photoperiod (Pp). All treatments grown in controlled environment chambers at Lincoln University, New Zealand.

Genotype	Pp	Weeks (days) to V_{base}				Mean (±SEM[2])	Mean (±SEM[2])
		4	8	12	18		
Medea	8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	17	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Kleppe	8	8 (56)	6 (42)	6 (42)	-[1]	46.6 (4.6)	52.5 (6.7)
	17	10 (70)	-	-	-	70.0 (-)	
Grasslands Nui	8	6 (42)	6 (42)	8 (56)	-	46.6 (4.6)	47.6 (3.4)
	17	6 (42)	8 (56)	-	-	49.0 (7)	
Grasslands Impact	8	4 (28)	2 (14)	4 (28)	-	23.3 (4.6)	37.3 (10.0)
	17	4 (28)	6 (42)	12 (84)	-	51.3 (16.8)	

[1]- = treatment combination did not achieve 50% of plants flowering.
[2]. = Standard error of the mean for weeks

4.3.6 Pattern of primordium production

4.3.6.1 Non-reproductive plants

No 'Medea' plants remained vegetative following exposure to constant 18°C, 17 h Pp, and thus 'Medea' is not included in the non-reproductive analysis. For 'Kleppe', Grasslands Nui' and 'Grasslands Impact', treatments are only included if they did not achieve 50% of plants flowering. In some treatments these genotypes had low numbers of reproductive plants and these were removed from this analysis.

For 'Kleppe', Grasslands Nui' and 'Grasslands Impact', accumulated organ number, defined as the sum of leaves and undifferentiated primordium, was expressed relative to HS (Figure 4.8). The relationship was conservative among genotypes and organ

accumulation could be described using a two straight line model utilising a breakpoint at HS 7.37 (±0.14) (Figure 4.8). Following emergence and until at HS7.37, organs were produced at 2.17 (±0.04) per HS, with a calculated y intercept of 1.8. Thereafter, primordium production slowed to constant 0.96 (±0.02) per HS (Figure 4.8). This relationship provides a mechanism for vegetative plants to maintain a constant number of organs on the stem apex as the HS increases. This model can be used to predict the number of organs on the stem apex for any given HS, prior to FI only, where HS can be estimated from daily temperatures and phyllochron.

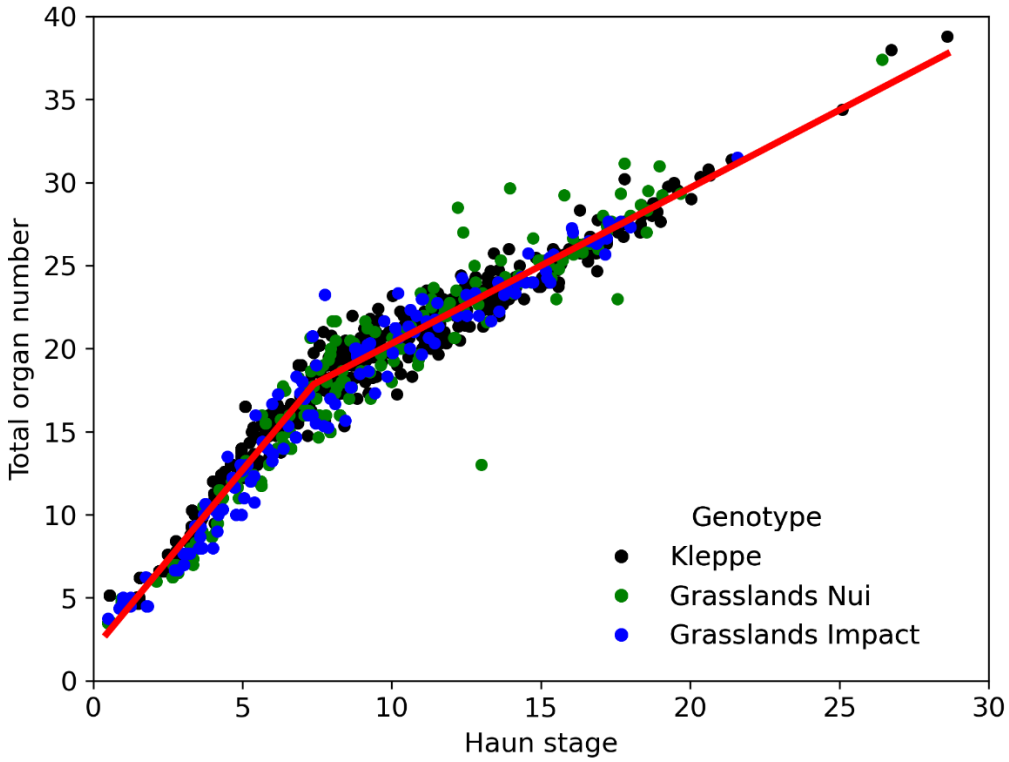


Figure 4.8. Relationship between total number of organs (primordium and emerged leaves) and Haun stage of vegetative perennial ryegrass for four genotypes grown in controlled environment chambers at Lincoln University, New Zealand experiments 5-12. Data points are the mean of 3-5 plants dissected weekly. For x less than 7.37 $\mathrm{y} = \mathrm{S1} \times x + \mathrm{y0} - \mathrm{S1} \times x0$, for x greater than 7.37; $\mathrm{y} = \mathrm{S2} \times x + \mathrm{y0} - \mathrm{S2} \times x0$. Where breakpoint x, $(x0)$= 7.37, $(\mathrm{y0})$= 17.8, slope line 1 $(\mathrm{S1})$= 2.17, slope line 2 $(\mathrm{S1})$= 0.96, R2 = 0.96.

4.3.6.2 Reproductive plants

When the organ number/HS deviates from the vegetative model (Section 4.3.6.1), plants become reproductive, and fitting three straight lines allows the timing of FI and TS to be calculated (Figure 4.2).

For 'Medea', all treatments went reproductive and conformed to this three straight line model (Figure 4.9). When grown in 17 h Pp, 'Medea' produced a similar pattern to the constant 18°C regardless of temperature imposed or duration of each treatment (Figure 4.9B,D,F,G). Most 17 h Pp treatments reached FI at ~HS 5 and TS at ~HS 7. The response was consistent for both 4°C treatments grown at either 8 (Figure 4.9G) or 17 h Pps (Figure 4.9H) where temperature limited leaf appearance and FI occurred at ~HS 5. When grown at 8, 12 and 18°C in 8 h Pp (Figure 4.9A,C,E), an increase in the duration of treatment conditions showed an increase in the HS at which FI occurred (FI^{HS}). For example, FI^{HS} increased from 6.8 to 13 for 'Medea' when primary induction increased from 2 – 12 weeks at 18°C (Figure 4.9A). Thus, 'Medea' showed a delayed FI in response to extended exposure to 8 h Pp but continued to produce leaves as the apex maintained a 'vegetative state' (Figure 4.4). Organ production continued in response to an increase in HS and FI occurred soon after transfer to the post treatment conditions. Thus, 'Medea' showed no change in the FI^{HS} in 17 h Pp while 8 h Pp delayed the FI^{HS}, suggesting that Pp is the driver of reproductive development for 'Medea'.

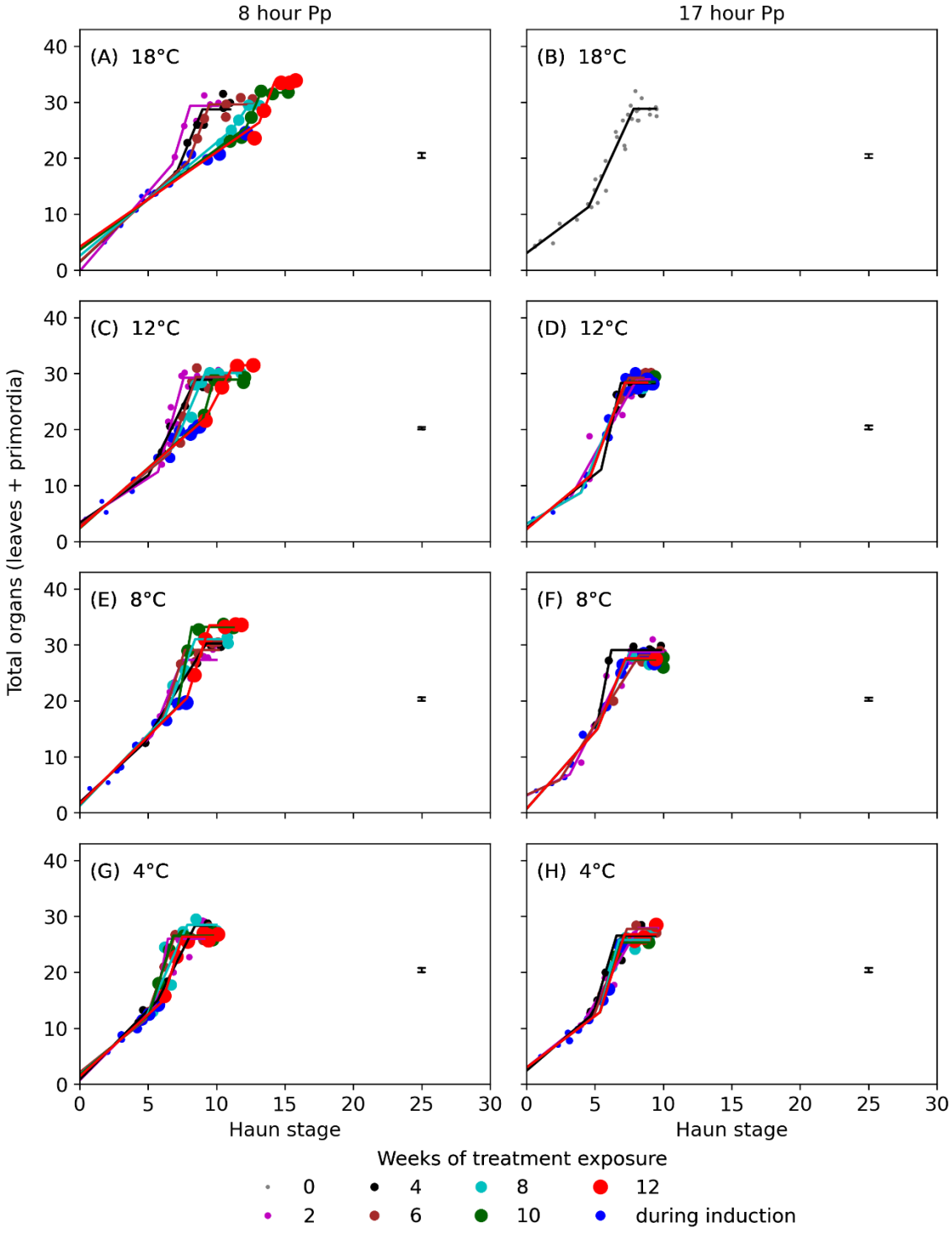


Figure 4.9. Accumulated number of organs relative to Haun Stage of genotype 'Medea' following vernalisation at four temperatures in either 8 or 17 hour photoperiod (Pp) for up to 12 weeks, followed by transfer to 18°C, 17 hour Pp. Grown in controlled environment chambers at Lincoln University, New Zealand. Error bar is the SEM for final assessment timing at the 12 week duration.

For ‘Kleppe’ no treatments went reproductive at 18°C (Figure 4.10 A, B). For both 8 and 12°C, no induction occurred for treatments with a 17 h Pp (Figure 4.10 D, F) while in 8 h Pp, flowering occurred in all treatments exposed to at least six weeks of treatment (Figure 4.10 C, E). Thus, in 8 h Pp, vernalisation was accelerated by short Pp and cool temperatures. At 4°C, induction showed a similar pattern requiring 8 weeks in 8 h Pp or 10 weeks in 17 hr Pp (Figure 4.10G,H). Overall the earliest FI^{HS} was 7.9 when treatment conditions were 4°C for 10 weeks, a result consistent for both 8 and 17 h Pp.

At 12°C, ‘Kleppe’ showed two response for delaying FI. Firstly, the same mechanism was shown as ‘Medea’ where continued exposure to 8 h Pp delayed FI (Figure 4.10C). Secondly, the ability to delay FI following transfer from treatment conditions. For example, following six weeks exposure to 12°C, 8 h Pp, FI was reached at HS12 when HS on transfer was ~7. This suggests either more long days were required to trigger FI or vernalisation was completed at a slower rate in the long Pp under post treatment conditions.

Overall, ‘Kleppe’ demonstrated a clear vernalisation requirement for cool temperatures and/or short Pp.

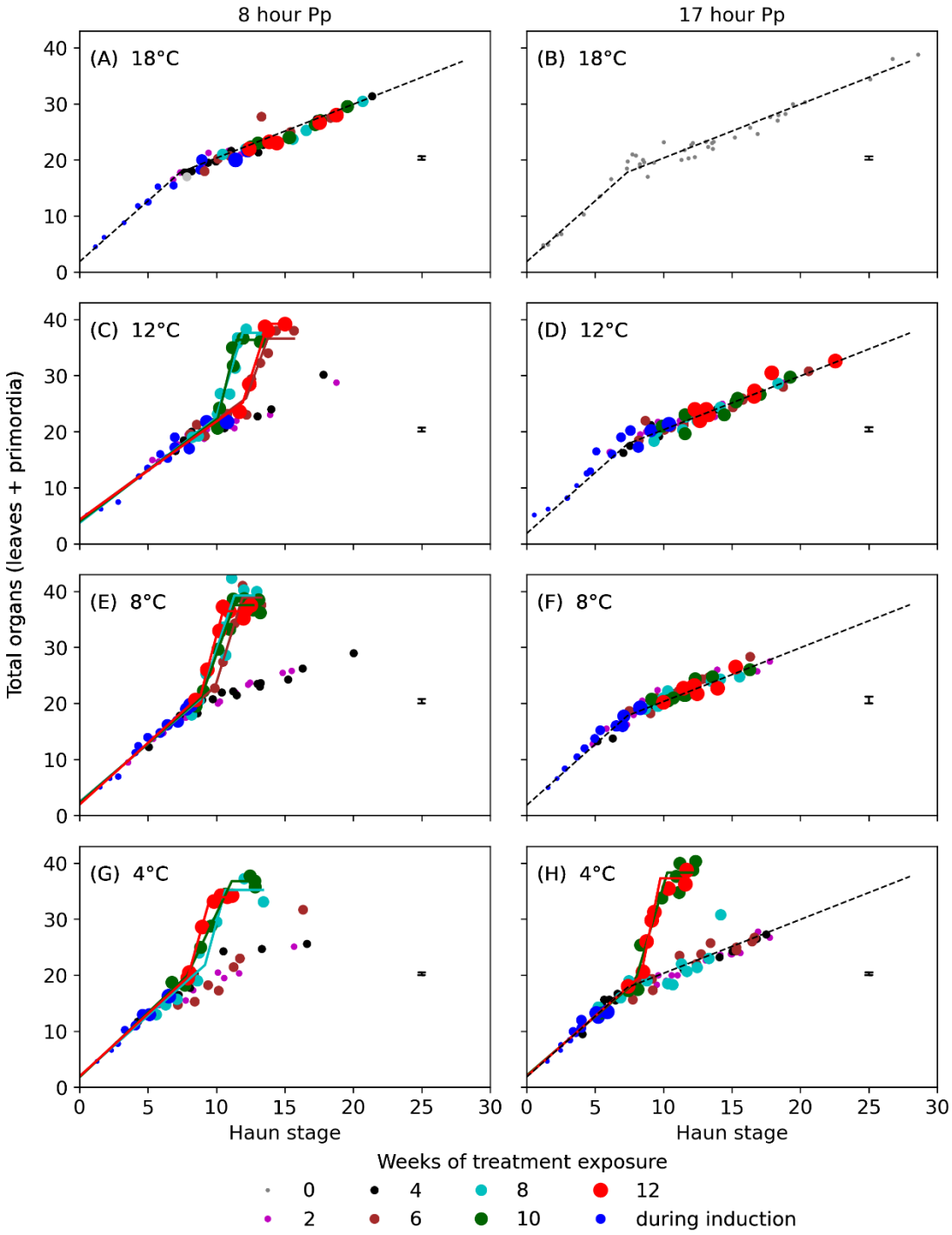


Figure 4.10. Accumulated number of organs relative to Haun Stage of genotype 'Kleppe' following vernalisation at four temperatures in either 8 or 17 hour photoperiod (Pp) for up to 12 weeks, followed by transfer to 18°C, 17 hour Pp. All grown in controlled environment chambers at Lincoln University, New Zealand. Error bar is the SEM for final assessment timing at the 12 week duration. Dashed line is the non reproductive model of breakpoint, x= 7.37, y= 17.8, slope line 1 = 2.17, slope line 2 = 0.96, R^2 = 0.96.

For ‘Grasslands Nui’, no treatments went reproductive when temperatures were kept at 18°C regardless of Pp (Figure 4.11A, B), a result repeated at 12°C in 17 h Pp (Figure 4.11D). However, FI occurred following eight weeks of exposure to 12°C, 8 h Pp. Thus, at 12°C, an 8 h Pp was required to induce flowering. At 8°C, induction was consistent when exposure occurred under both 8 and 17 h Pp, but generally FI occurred at a lower HS when exposed to 8 h Pp (Figure 4.11E,F). At 4°C, all treatments that were exposed to 6 weeks or longer reached FI (Figure 4.11G,H) at HS 6.6 ±0.39.

For ‘Grasslands Impact’ no treatments went reproductive when primary induction occurred at 18°C and organ production closely followed the vegetative model (Figure 4.12A, B). When exposed to 12°C, FI occurred after 4 weeks exposure in 8 h Pp (Figure 4.12C) while in 17 h Pp, FI occurred after 12 weeks exposure (Figure 4.12D). In 17 h Pp ~25% of plants flowered after four weeks duration but only reached 50% after 12 weeks (Figure 4.7). Thus, several treatments diverged from the vegetative model but were not included in reproductive analysis (Figure 4.12D). At 8°C, all treatments tested reached FI when primary induction occurred in an 8 h Pp with the lowest FI^{HS} achieved after six weeks exposure (HS ~5.5) (Figure 4.12E). Treatment durations of two and four weeks produced extra leaves following transfer into post treatment conditions and reached a FI^{HS} of ~9. In 17 h Pp (8°C) six weeks exposure was required to achieve FI and the shortest time to FI was 5 HS after eight weeks exposure (Figure 4.12F). At 4°C, durations of four weeks or more induced FI in both 8 and 17 h Pp (Figure 4.12G,H) with similar FI^{HS} (8 h Pp mean = HS 5.4 (±0.16), 17 h Pp mean = HS 5.3 (±0.32)).

Overall, ‘Grasslands Nui’ showed an increased requirement for vernalisation from cool temperatures and short Pp than ‘Grasslands Impact’.

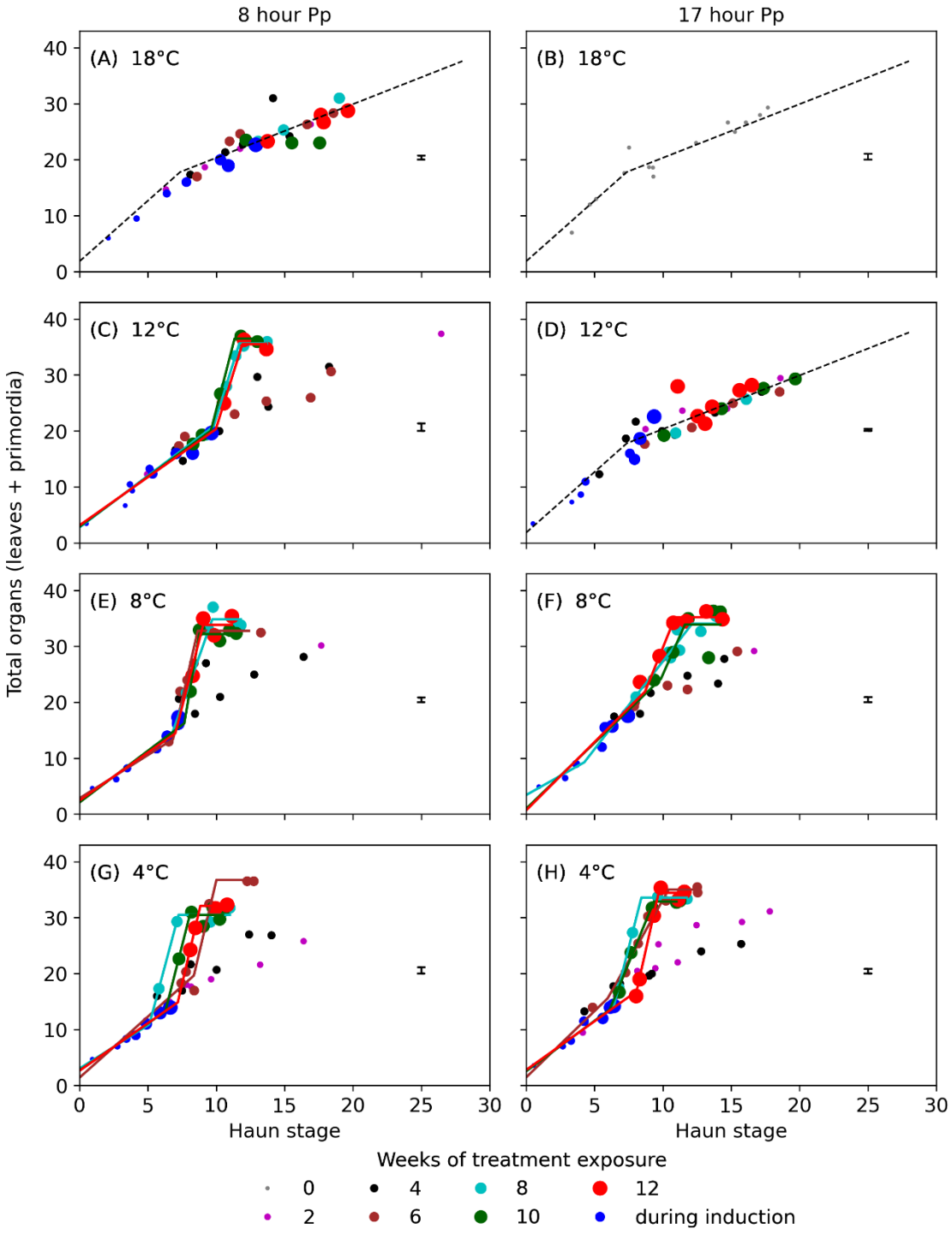


Figure 4.11. Accumulated number of organs relative to Haun Stage of genotype 'Grasslands Nui' following vernalisation at four temperatures in either 8 or 17 hour photoperiod (Pp) for up to 12 weeks, followed by transfer to 18°C, 17 hour Pp. All grown in controlled environment chambers at Lincoln University, New Zealand. Error bar is the SEM for final assessment timing at the 12 week duration. Dashed line is the non reproductive model of breakpoint, x= 7.37, y= 17.8, slope line 1 = 2.17, slope line 2 = 0.96, R^2 = 0.96.

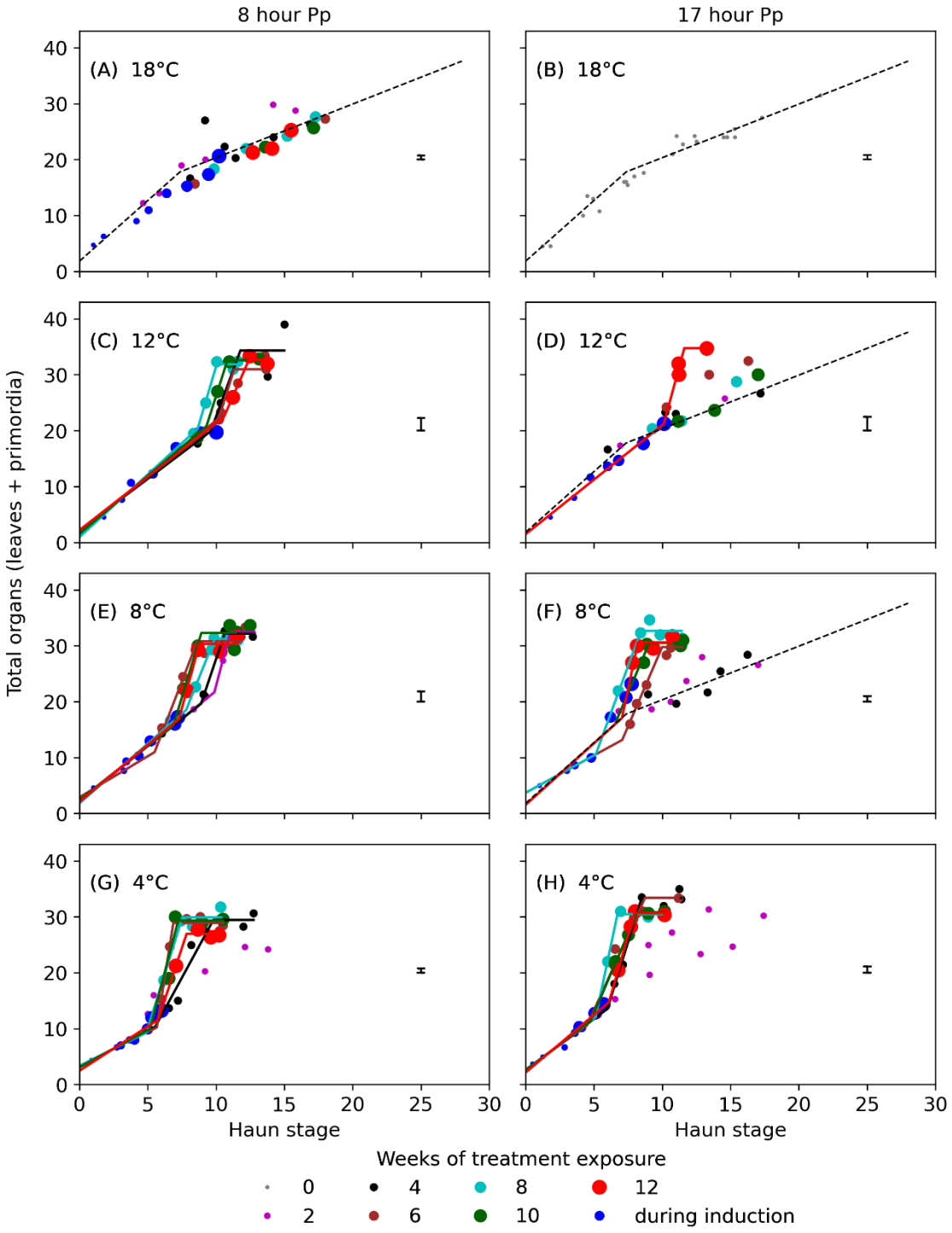


Figure 4.12. Accumulated number of organs relative to Haun Stage of genotype 'Grasslands Impact' following vernalisation at four temperatures in either 8 or 17 hour photoperiod (Pp) for up to 12 weeks, followed by transfer to 18°C, 17 hour Pp. All grown in controlled environment chambers at Lincoln University, New Zealand. Error bar is the SEM for final assessment timing at the 12 week duration. Dashed line is the non reproductive model of breakpoint, x= 7.37, y= 17.8, slope line 1 = 2.17, slope line 2 = 0.96, R^2 = 0.96.

4.3.6.3 Combined vegetative and reproductive model

Few treatments produced sufficient leaves prior to FI to be analysed utilising a combination of the 'vegetative model' followed by an increase in organ number at FI. Those that did were generally treatments at the warmest temperature and longest exposure durations where FI occurred for individual genotypes following 8 h Pp, e.g. 12°C for 'Kleppe' (Figure 4.13C,D). For 'Medea' at 18°C (8 h Pp) (Figure 4.13A,B), 'Kleppe' (Figure 4.13C,D) and 'Grasslands Impact' (Figure 4.13G,H) at 12°C (8 h Pp) the vegetative model fitted data well until FI. However, for 'Grasslands Nui' the fit was weaker around the 'vegetative model' breakpoint (HS 7), largely due to a lack of data points.

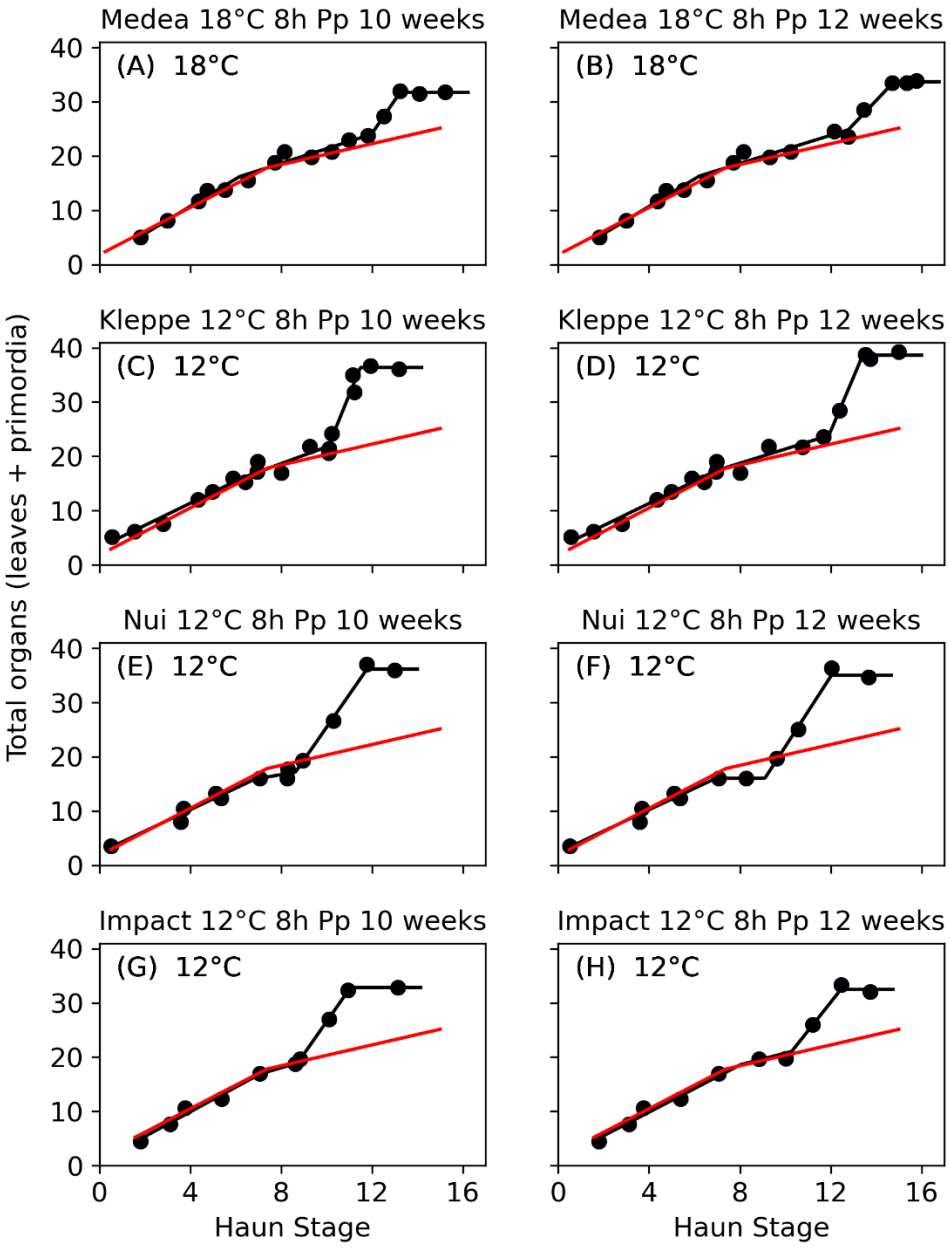


Figure 4.13. Four line model of the number of organs relative to Haun Stage of four perennial ryegrass genotypes at different temperatures, in 8 h photoperiod (Pp) after either 10 or 12 weeks of vernalisation. These conditions incorporate vegetative growth following induction to flower, followed by reproductive growth after transfer to 18°C 17 h Pp. All combinations grown in controlled environment chambers at Lincoln University, New Zealand. Red line is the non reproductive model of breakpoint, x= 7.37, y= 17.8, slope line 1 = 2.17, slope line 2 = 0.96, R^2 = 0.96

Overall, the mean of the regression slopes during the vegetative phase was constant between genotypes (Table 4.3) while during the ear development phase 'Medea' generally produced fewer primordium/HS (Figure 4.14).

Table 4.3. Slope of the regression lines for the vegetative and reproductive phases fitted to the number of organs (total leaves, spikelets and primordium) and Haun stage relationship for four perennial ryegrass genotypes grown in temperatures of 4,8,12 and 18°C under 8 or 17 hour photoperiod in controlled environment chambers at Lincoln University, New Zealand**.**

Genotype	Vegetative slope	SEM	Reproductive slope	SEM	count
Medea	2.07	0.07	6.91	0.36	43
Kleppe	2.06	0.05	8.23	0.48	13
Grasslands Nui	1.84	0.07	8.45	0.65	18
Grasslands Impact	1.84	0.06	7.83	0.47	26
Grand mean	1.97	0.04	7.60	0.24	

Note: Grand means are different to the mean of the genotype data presented due to differences in number of treatments reproductive within a genotype.

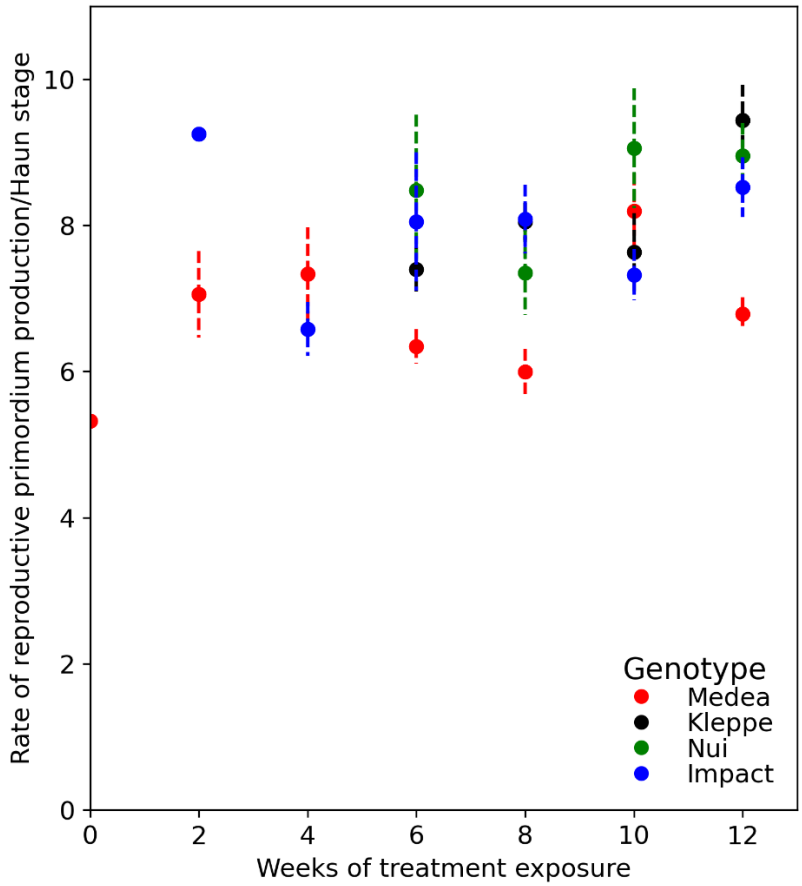


Figure 4.14. Rate of primordium production per Haun stage between floral initation and terminal spikelet of four perennial ryegrass genotypes. Data points are the mean of all repoductive plants induced to flower following exposure to either 4,8,12 or 18°C in in either 8 or 17 hour photoperiod for up to 12 weeks and subsequently transferred to constant 18°C, 17 hour Pp. FI occurred following transfer for all data points. Lines represent the SEM. All treatments grown in controlled environment chambers at Lincoln University, New Zealand.

4.3.6.4 Time from floral initiation to terminal spikelet

No treatment exposed to 8 h Pp reached FI until transferred into post treatment conditions of 17 h Pp. Therefore, in this experiment Pp had no influence on the duration from FI to TS. The duration from FI to TS was conservative among genotypes and lasted approx. 2 Haun stages for all treatments (Figure 4.15). The mean of all data was 2.24 (±0.11) while regression analysis described the relationship as TS^{HS} = 0.84 (±0.043)×FI^{HS}+3.36 (±0.34) (R^2=0.97).

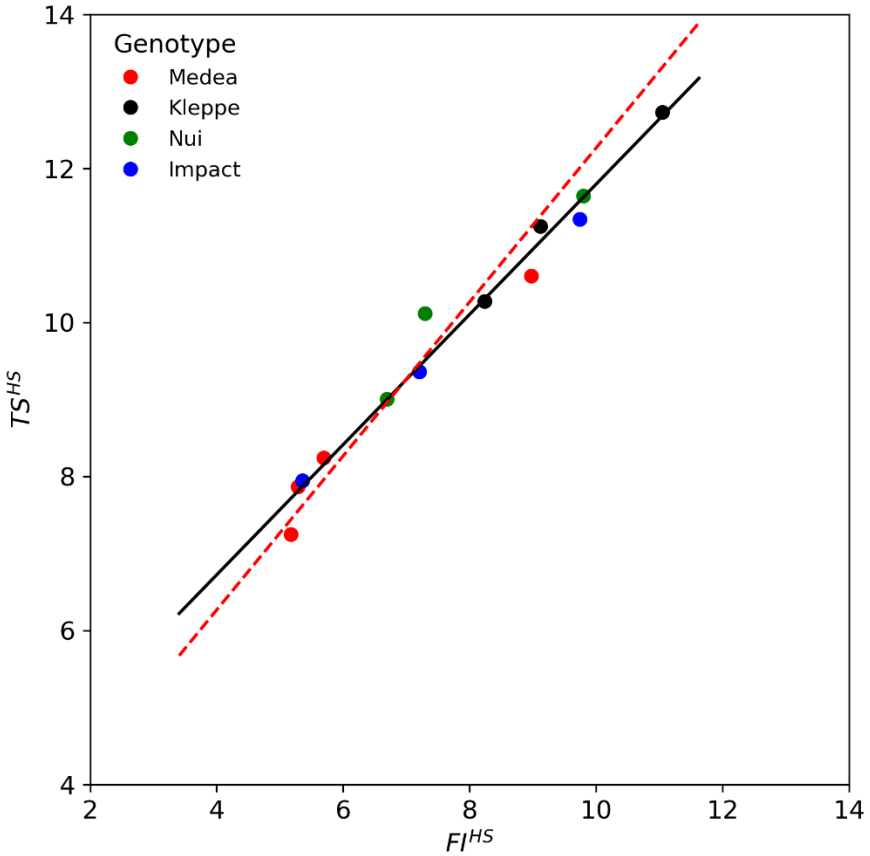


Figure 4.15. Time, represented as Haun stage, from floral initiation Haun stage (FI^{HS}) to terminal spikelet Haun stage (TS^{HS}) of four perennial ryegrass genotypes following vernalisation and transfer to constant 18°C 17 hour photoperiod when grown in controlled environment chambers at Lincoln University, New Zealand. Each data point is the mean of two photoperiods and up to 7 durations, data only included if more than 50% of plants were reproductive within a treatment. Regression (black line), TS^{HS} = 0.84 (±0.043)×FI^{HS}+3.36 (±0.34) (R^2=0.97), Red line is the means of the TS^{HS} – FI^{HS} where TS^{HS} = FI^{HS} + 2.24 (±0.11)

4.3.7 Leaves to appear after TS Haun stage

Following terminal spikelet, there was a mean of 2.15 (±0.06) leaves remaining to emerge from the leaf sheath. This was quantified as FLN = 0.90 (±0.05) ×TS^{HS} + 3.05 (±0.47) (R^2 = 0.94) when data were separated into genotypes and the mean of each temperature by Pp treatment (Figure 4.16A). The relationship was described as FLN =1.00 (±0.09) × TS^{HS} + 2.01 (±0.90) (R^2 = 0.94) when data were expressed as the mean of temperature and Pp

during vernalisation, assuming no genotype response (Figure 4.16B). In practical terms all three models give similar results and the simplest outcome would be to assume FLN =TS^{HS} + 2.01 or FLN = TS^{HS} + 2.15 (Figure 4.17). For ease of use, FLN =1.00 (±0.09) ×TS^{HS} + 2.01 (±0.90) can be shortened to FLN =TS^{HS} + 2.01 and will be used in this chapter.

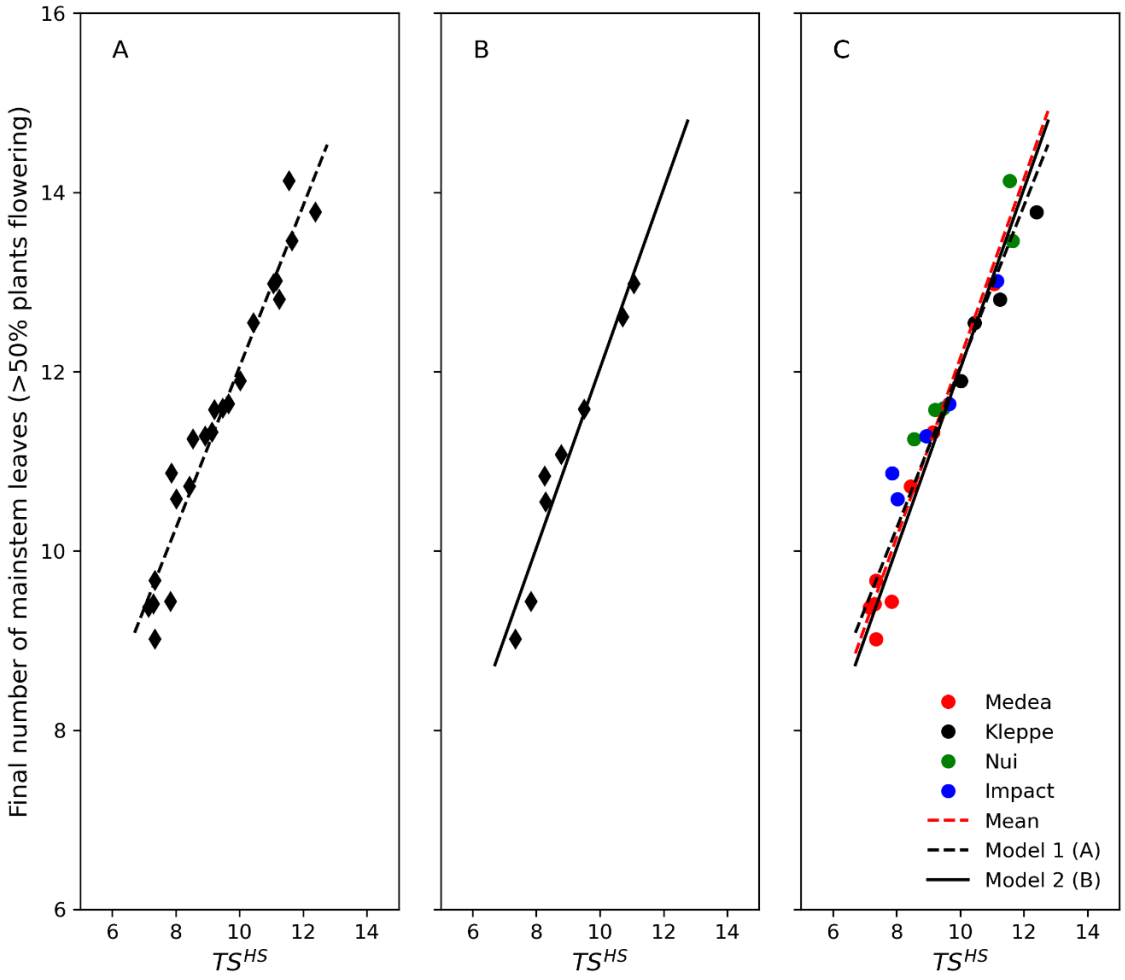


Figure 4.16. The number of leaves to emerge against terminal spikelet Haun stage (TS^{HS}) where 50% of plants flowered in four genotypes of perennial ryegrass following vernalisation and transfer to constant 18°C ,17 hour photoperiod (Pp). All treatments grown in controlled environment chambers at Lincoln University, New Zealand. 'A' is the mean of data grouped by genotype, temperature and Pp, n = 22, FLN = 0.90 (±0.05) ×TS^{HS} + 3.05 (±0.47) (R^2 = 0.94), B is grouped by temperature and Pp, n = 8, FLN =1.00 (±0.09) ×TS^{HS} + 2.01 (±0.90) (R^2 = 0.94). 'C' compares the models from A and B with the mean of FLN minus the TS^{HS} where FLN = TS^{HS} + 2.15 (±0.07) represented as the "Mean", plotted data are from 'A'.

4.3.8 Final number of leaves

The final number of main stem leaves (FLN) gives an indication of how the treatment conditions influenced the time to final leaf emergence. Since the time from FI until final leaf emergence was constant at 4 leaves, FLN indicates when FI occurred.

'Medea' produced ~9 main stem leaves when treatment conditions occurred in 17 h Pp. This was consistent across all temperatures tested (Figure 4.17B,D,F,H). When exposed to 8 h Pp, the FLN increased relative to the increased Tt accumulated, concluding with 10 leaves after 12 weeks at 4°C and increasing to 16 leaves after 12 weeks in 18°C (Figure 4.17A,G)

For 'Kleppe', FLN was constant when treatments occurred at 4°C in 17 h Pp (Figure 4.17B), whereas in 8 h Pp a reduction from 14 to 11 leaves occurred as the duration increased from eight to 12 weeks (Figure 4.17A). At 8°C, a reduction of one leaf occurred between six and 12 weeks of primary induction (Figure 4.17C), no induction occurred in 17 h Pp. At 12°C, FLN decreased from ~16 after six weeks until a minimum of ~13 at V_{sat} (8-10 weeks) followed by an increase to 15 with 12 weeks exposure to 8 h Pp (Figure 4.17E).

At 4°C, 'Grasslands Nui' showed a reduction in FLN from ~13, following six weeks exposure to a constant of ~11 leaves after 12 weeks in both 8 and 17 h Pp (Figure 4.17A,B). At 8°C, FLN was constant when treatments occurred under 17 h Pp, while in 8 h Pp a reduction from ~13 to 11 leaves occurred as exposure duration increased from 6 to 10 weeks. FLN at 12°C reduced to a minimum at 10 weeks (Figure 4.17E).

For 'Grasslands Impact', at 4°C FLN reduced to a minimum of ~10 after six weeks, in 8 h Pp, or eight weeks in 17 h Pp (Figure 4.17A,B). At 8°C, minimum FLN was ~10 after 12 weeks in exposure in 17 h Pp or eight weeks in 8 h Pp where FLN increased following further exposure. The reduction followed by increase pattern continued at 12°C where 15 leaves were produced after four weeks, 11 following eight weeks increasing to 14 leaves after 12 weeks of treatment exposure (Figure 4.17E).

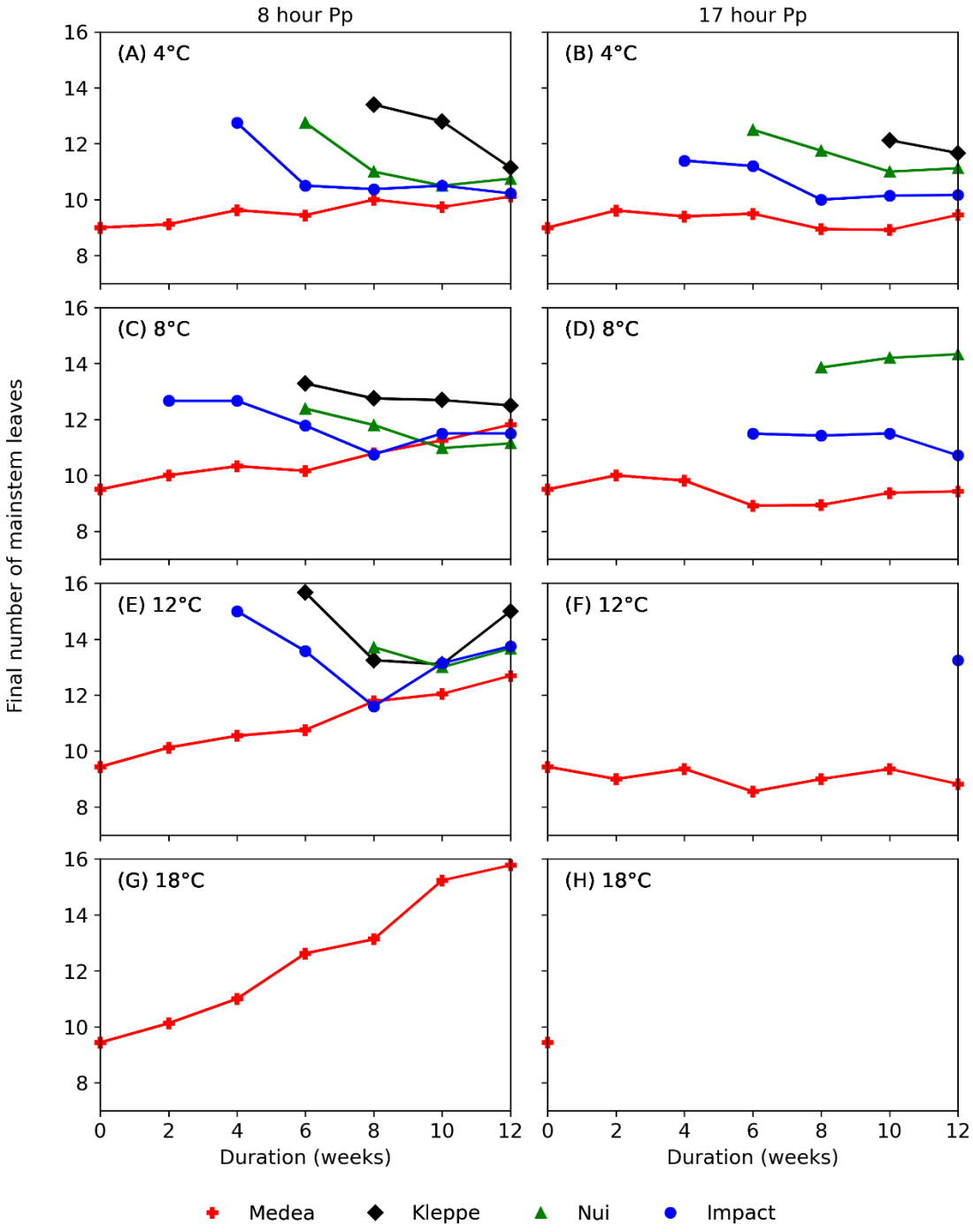


Figure 4.17. Final number of main stem leaves for treatments that obtained flowering in four genotypes of perennial ryegrass following vernalisation of up to 12 weeks in 8 or 17 hour photoperiod (Pp) and transferred to constant 18°C, 17 hour photoperiod when grown in controlled environment chambers at Lincoln University, New Zealand.

4.3.8.1 Leaves produced post transfer.

Leaf production post transfer (LN_{PT}) from treatment exposed to 8 h Pp into constant 18°C, 17 h Pp gives an indication of the plants ability to respond to long Pp during secondary induction. A minimum LN_{PT} suggests vernalisation had been fully saturated and therefore flowering proceeds in the shortest time possible.

Most treatments conformed to produce approximately 4 leaves post V_{sat}. This was most consistent in treatments which had an 8 h Pp during PI (Figure 4.18A,C,E), where no FI occurred prior to transfer into post treatment conditions.

When 'Medea' was grown in 8 h Pp, the LN_{PT} reduced from ~6 after two weeks treatment exposure to 4, generally after six weeks (Figure 4.18A,C,E). In 17 h Pp, the LN_{PT} reduced (Figure 4.18B,D,F) as the reproductive stage at transfer increased (Figure 4.4), but LN_{PT} reduced towards 4 in a similar response to 8 h Pp for those treatments where FI was not visually detected prior to transfer. For example, at 4°C 'Medea' produced 5 LN_{PT} with 6 weeks of exposure (Figure 4.18B) where no visible change in apex state was recorded at transfer (Figure 4.4B). At 8 and 12°C, the final leaf had emerged at transfer to post treatment conditions (Figure 4.18D,F). Following transfer from 8 h Pp, to 17 h Pp, FI in 'Medea' was often observed within 2 days and was calculated to occur within 1 HS.

In 8 h Pp, LN_{PT} for 'Kleppe' reduced from more than 8 at V_{base} to conform with 4 LN_{PT} as the duration of vernalisation increased towards V_{sat} in temperatures of 4, 8 and 12°C (Figure 4.18A,C,E). In 17 h Pp, the LN_{PT} at 4°C was similar for durations from 10–12 weeks (Figure 4.18B).

For 'Grassland Nui' grown in 8 h Pp, LN_{PT} reduced from ~7 at V_{base} to conform with 4 LN_{PT} as the duration of treatment exposure increased to 10 weeks at temperatures of 4, 8 and 12°C (Figure 4.18A,C,E). A similar response was shown at 4°C in 17 hr Pp (Figure 4.18B) while at 8°C LN_{PT} remained relatively constant at ~8 for all durations where V_{base} was achieved (Figure 4.18D).

For 'Grasslands Impact', LN_{PT} reduced in 8 h Pp from more than 9 at V_{base}, to conform with the 4 LN_{PT} as the duration of treatment exposure increased towards V_{sat} at 12 (4°C), 10

(8°C) and eight (12°C) weeks (Figure 4.18A,C,E) (Table 4.4). V_{sat} was defined as the duration of treatment exposure to reduce the number of leaves produced post transfer to less than 4.75.

Overall there was a repeatable pattern that when genotypes had undergone primary induction in short days, and were subsequently transferred to long days, there was a decline in the LN_{PT} as the duration of treatment exposure increased (Figure 4.18).

Following V_{base}, 'Grasslands Nui', 'Grasslands Impact' and 'Kleppe' all produced a facultative response where the HS from transfer to FI^{HS} decreased as vernalisation continued, and they progressed towards V_{sat}. This provides a mechanism for the reduction in LN_{PT} (Figure 4.18).

Table 4.4. Duration (weeks) required to reach saturation of the vernalisation response for four perennial ryegrass genotypes grown in four temperatures in 8 or 17 hour photoperiod for up to 12 weeks before transfer to constant 18°C 17 h Pp. All treatments grown in controlled environment chambers at Lincoln University, New Zealand.

Genotype	Pp (hours)	Temperature (°C)				Mean (±SEM)
		4	8	12	18	
Medea	8	10	6	6	4	6.5 (1.25)
	17	10	6	6	-*	7.3 (1.3)
Kleppe	8	12	12	10	-	11.3 (0.6)
	17	12	-	-	-	12 (-)
Grasslands Nui	8	10	10	10	-	10 (0)
	17	10	8	-	-	9 (1.0)
Grasslands Impact	8	12	10	8	-	10 (1.2)
	17	8	12	12	-	10.6 (1.3)

* did not flower or did not spend time in primary induction.

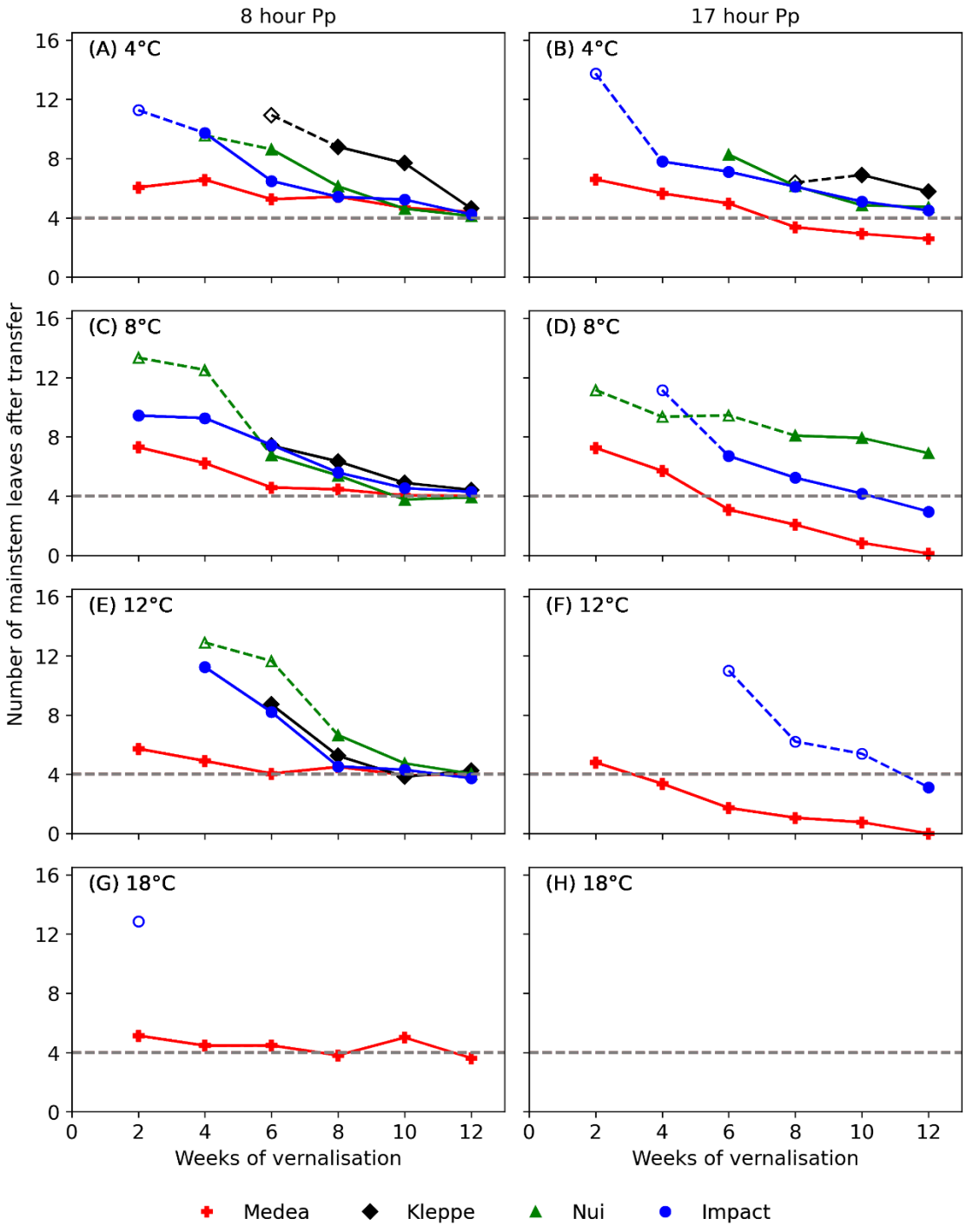


Figure 4.18. Number of leaves produced on reproductive stems of treatments that achieved >50% of plants flowering (closed symbols) following transfer to constant 18°C, 17 hour photoperiod (Pp) conditions of four perennial ryegrass genotypes vernalised at four temperatures under 8 or 17 hour Pp. All grown in controlled environment chambers at Lincoln University, New Zealand. Open symbols with dashed line are the mean of reproductive plants where less than 50% plants flowered, dashed grey line = constant 4 leaves for reference.

4.3.9 Rate of vernalisation

V_{sat} was calculated as the duration of treatment conditions to reduce the number of leaves post transfer to less than 4.75. Three or more data points allowed the fitting of linear regression to all genotypes for 'Medea' and 'Grasslands Impact' in both 8 h Pp and to 17 h Pp. In 17 h Pp, V_{sat} was determined as the treatments which produced the lowest final number of leaves. The slope of the regression is the average rate of progress towards V_{sat} where, when the sum of daily values reaches unity, the vernalisation response is saturated.

For 'Medea', the duration required to reach V_{sat} was the same for both 8 and 17 h Pp treatments but was different between 4, 8 and 12°C, where the rate towards V_{sat} was 0.0014/°C (±0.0003).

'Kleppe' required the full 12 weeks exposure (84 days) at 4°C to reach V_{sat} in both 8 and 17 h Pp (the only 'Kleppe' temperature treatment reproductive in 17 h Pp). In 8 hr Pp, the rate towards V_{sat} for 'Kleppe' was 0.0003/°C (±0.0002) (Figure 4.19). However, the regression P value was 0.333 suggesting the slope is not significant. Thus, all further analysis for 'Kleppe' assumed no difference in efficacy between 4 and 12°C. Thus, a constant accumulation of 0.0127 (±0.00079) units/day at temperatures between 4 and 12°C is appropriate.

In 8 hr Pp, 'Grasslands Nui' reached V_{sat} after 10 weeks at all temperatures. The regression also derived a flat line with an intercept of 0.0143/day. In 17 h Pp, the 4°C treatment required 10 weeks duration to achieve (V_{sat}) while at 8°C saturation was achieved in eight weeks.

For 'Grasslands Impact' in 8 h Pp, there was a trend (P=0.07) for the rate of vernalisation to increase by 0.0007/°C (±0.00009) as the temperature increased from 4 to 12°C (Figure 4.19). However, in 17 h Pp the duration required increased as temperature increased from six weeks at 4°C to 12 weeks at both 8 and 12°C, thus the rate of progress decreased as temperature increased in 17 h Pp (Figure 4.19).

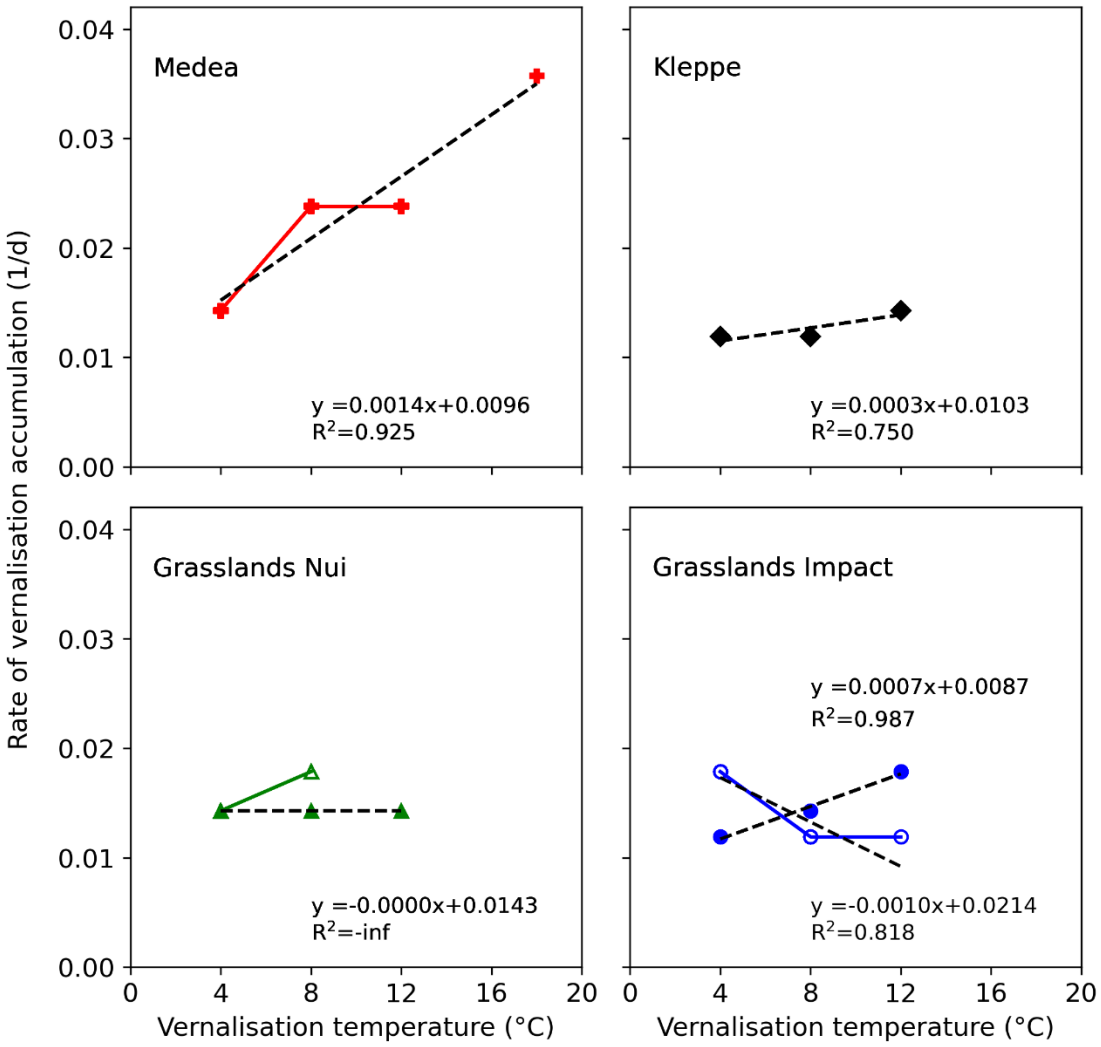


Figure 4.19. Rate of vernalisation, calculated as 1/days to saturate vernalisation under 8 (closed symbols) and 17 (open symbols) hour photoperiod, for four perennial ryegrass genotypes grown at four temperatures in controlled environment chambers at Lincoln University, New Zealand. Dashed line is the regression analysis for 8 h Pp only (and 17 h Pp in Grasslands Impact), coloured lines link 17 h Pp data which may directly overlay 8 hr Pp data e.g. 'Kleppe' at 4°C has data points that are identical at both Pps.

4.3.10 Influence of temperature on vernalisation effectiveness in 8 h Pp

The vernalisation effectiveness (V_{eff}) was as calculated as 1/days to reach V_{sat} (Table 4.4) for all temperatures and Pp where FI was achieved. For 'Medea' in 8 h Pp, the maximum effectiveness (V_{eff}) was at 18°C, reducing at lower temperatures (duration to V_{sat} increased, Table 4.4) and can be described as $V_{eff} = 0.0396x + 0.2679$ (R^2=0.93) for temperatures between 0 and 18°C. For both 'Grasslands Nui' and 'Kleppe', V_{eff} achieved a constant maximum between temperatures of 4 and 12°C, with an extrapolation (which requires experimental confirmation) fitted to reach zero at -4°C, thus crossing the y axis at 0.5 with a slope of 0.125. 'Grasslands Impact' had maximum V_{eff} at 12°C reducing as temperatures reduced to cross the y axis (0°C) at 0.49 where $V_{eff} = 0.0417x + 0.4889$ (R^2=0.98). Thus, effective vernalisation days or progress toward unity can be calculated from observed daily temperatures or accumulated daily fractions.

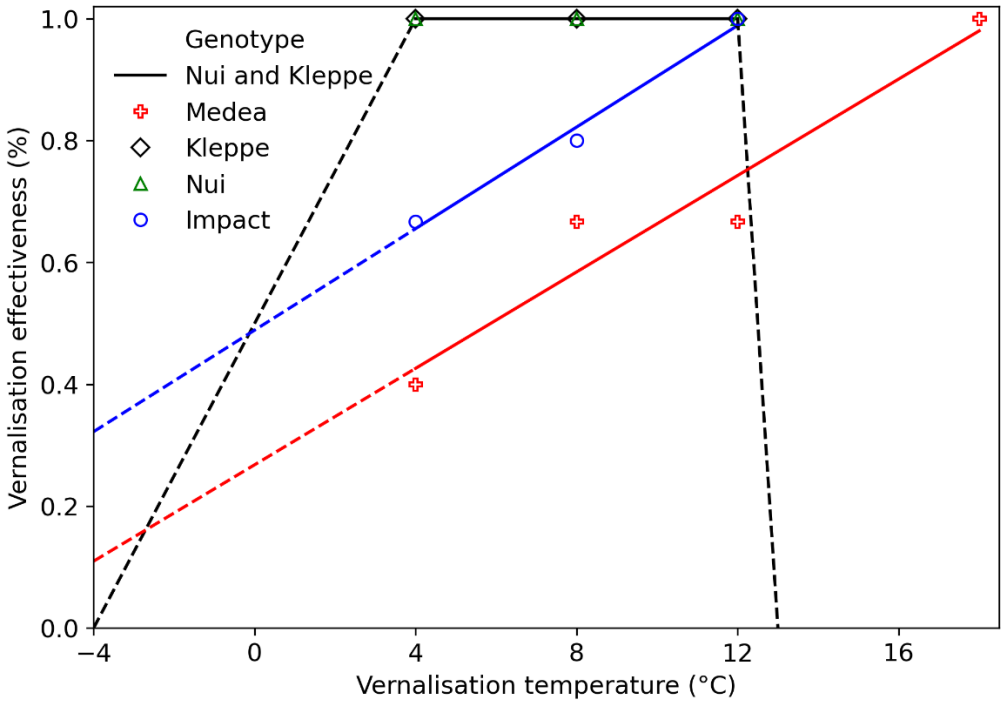


Figure 4.20. Effectiveness of temperatures between 4 and 18°C in an 8 hour photoperiod during vernalisation where a value of 1 is unity (maximum effectiveness) for four perennial ryegrass genotypes grown in controlled environment chambers at Lincoln University, New Zealand. Dashed lines are extrapolations outside the data set to a temperature of zero and require additional confirmation.

4.3.11 Predicting vernalisation progress

Prediction of vernalisation progress towards flowering requires two targets, V_{base} where 50% of plants achieve flowering, but flowering takes longer, and V_{sat}, where flowering occurs in the shortest timeframe e.g. Figure 2.4. V_{base} was calculated as the number of vernal days (Vdd) to achieve 50% of plants flowering while the number of vernal days at V_{sat} was accumulated from Section 4.3.8.1 (Table 4.4).

For 'Medea', V_{base} is zero Vdd, while V_{sat} is 28 Vdd in 8 h Pp increasing to 42 Vdd in a 17 h Pp (mean of all temperatures) (Figure 4.21A). However, the change in FLN for 'Medea' was small in 17 h Pp (Figure 4.17 B,D,F) and a V_{sat} value of 28 Vdd may be acceptable for both Pps.

For 'Kleppe', V_{base} increased from 46.7 to 70 Vdd as Pp increased from 8 to 17 h, V_{sat} increased from 70 to 84 Vdd as Pp increased from 8 to 17 h, ~1.5 days/h (Figure 4.21B).

Grasslands Nui' showed the same pattern as 'Kleppe' in 8 h Pp but days to V_{sat} reduced in 17 h Pp to 56 Vdd (Table 4.5, Figure 4.21C).

'Grasslands Impact' was intermediate of 'Medea' and 'Kleppe' in 8 h Pp with a V_{base} of 23.3 Vdd and a V_{sat} of 56 Vdd. In 17 h Pp, the V_{base} increased from 28 to 42 to 84 Vdd (mean 51) as temperature increased from 4 to 8 to 12°C respectively (Figure 4.21D). At 12°C, V_{sat} was the same as V_{base} as 12 weeks duration was the only treatment to achieve 50% flowering.

Overall, the mean of V_{base} in 8 h Pp was 26.9 (±6.01) Vdd and V_{sat} was 64.6 (±4.90) Vdd, giving a mean duration between these two stages of 37.7 Vdd. In 17 h Pp, the overall mean V_{base} was 32.2 (±10.0) Vdd and V_{sat} 65.3 (±5.7) Vdd, with a mean duration between these two stages of 33.1 Vdd. Thus, changes in the V_{base} of different genotypes was the main source of different responses among genotypes (Figure 4.21, Figure 4.2).

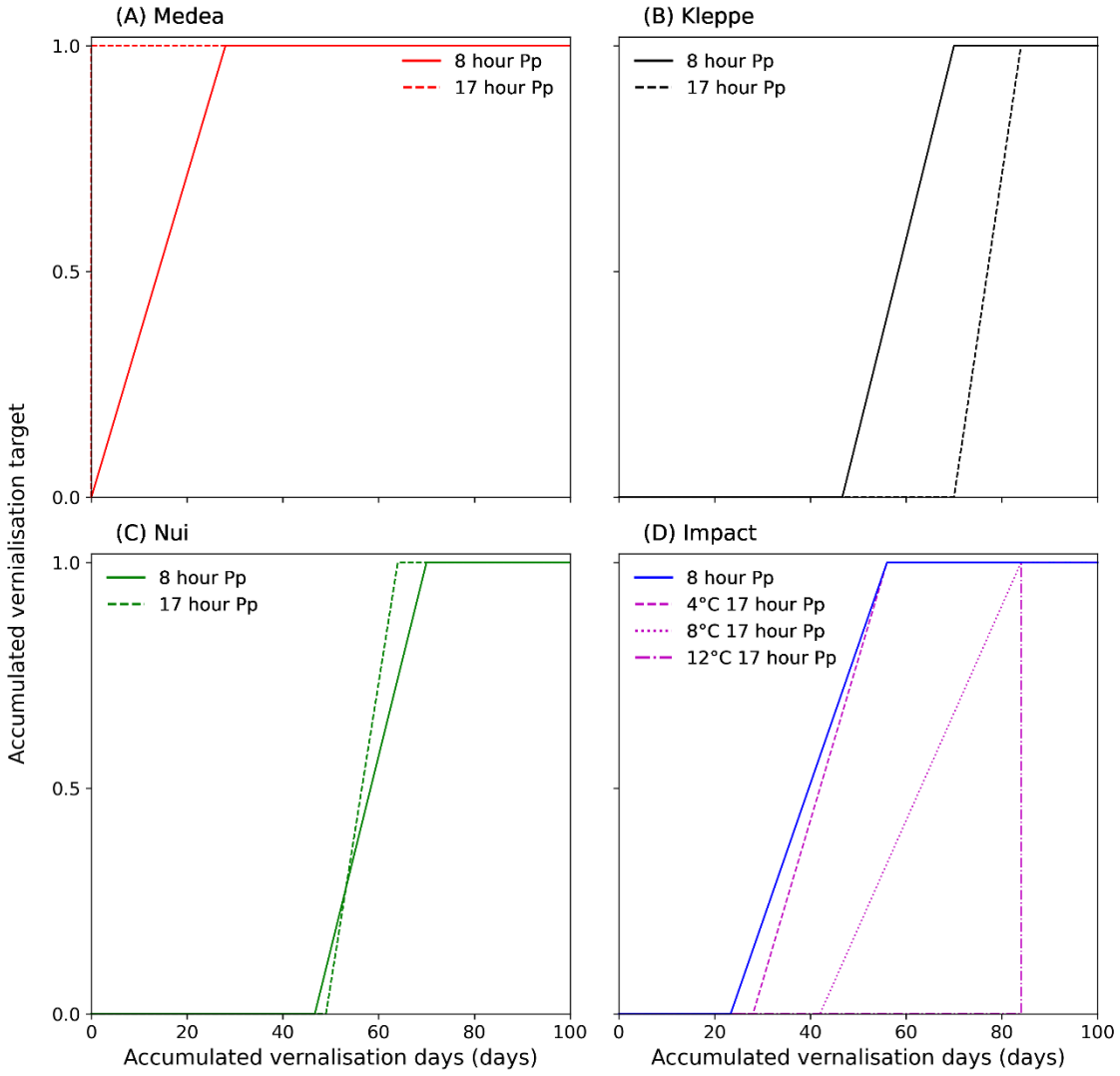


Figure 4.21. Graphic representation of the number of vernalisation days required for 50% of plants to achieve flowering (base vernalisation, $V_{base,}$ where lines depart from zero) and the days to saturate the vernalisation response (V_{sat}) of four perennial ryegrass genotypes grown in either 8 or 17 hour photoperiod (Pp) in controlled environment chambers at Lincoln University, New Zealand. Values of V_{base} are the means of treatments from 4, 8 or 12°C which achieved floral initiation.

Table 4.5. Calculated minimum number of vernal days (Vdd) required for 50% of plants to achieve flowering (base vernalisation, V_{base}) and days to saturate the vernalisation response (V_{sat}) of four perennial ryegrass genotypes grown in controlled environment chambers at Lincoln University, New Zealand. Values of V_{base} are the means of treatments form 4, 8 or 12°C which achieved floral initiation.

Genotypes r	V_{base} (Vdd)		V_{sat} (Vdd)	
	8 h Pp	17 h Pp	8 h Pp	17 h Pp
'Medea'	0	0	28	42
'Kleppe'	46.6	70	70	84
'Grasslands Nui'	46.6	49	70	56
'Grasslands Impact'[1]	23.3	51 (28-84)	56	75 (56-84)
Mean	21.1 (±11.2)	42.6 (±15)	56(±9.89)	67.2 (±6.87)

[1.] Values in parenthesis represent the range where days increased with increasing temperature

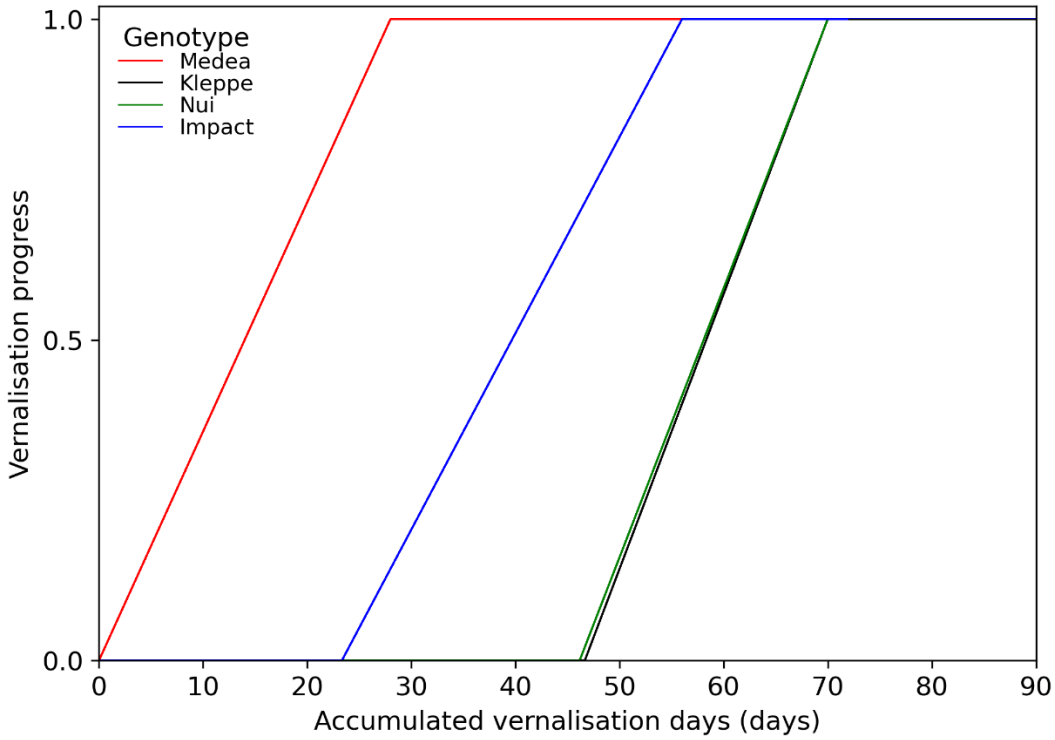


Figure 4.22. Graphic representation of the number of vernalisation days required for 50% of plants to achieve flowering (base vernalisation, $V_{base,}$ where lines depart from zero) and the days to saturate the vernalisation response (V_{sat}) of four perennial ryegrass genotypes grown in either 8 hour photoperiod (Pp) in controlled environment chambers at Lincoln University, New Zealand. Values of V_{base} are the means of treatments from 4, 8 or 12°C.

The increase in V_{base} reflected the latitude of origin and increased by approximately 2.6 Vdd per degree of latitude in both 8 and 17 h Pp (Figure 4.23). However, for the 8 h Pp this trend broke at ~50° latitude as 'Kleppe' required the same V_{base} as 'Grasslands Nui'.

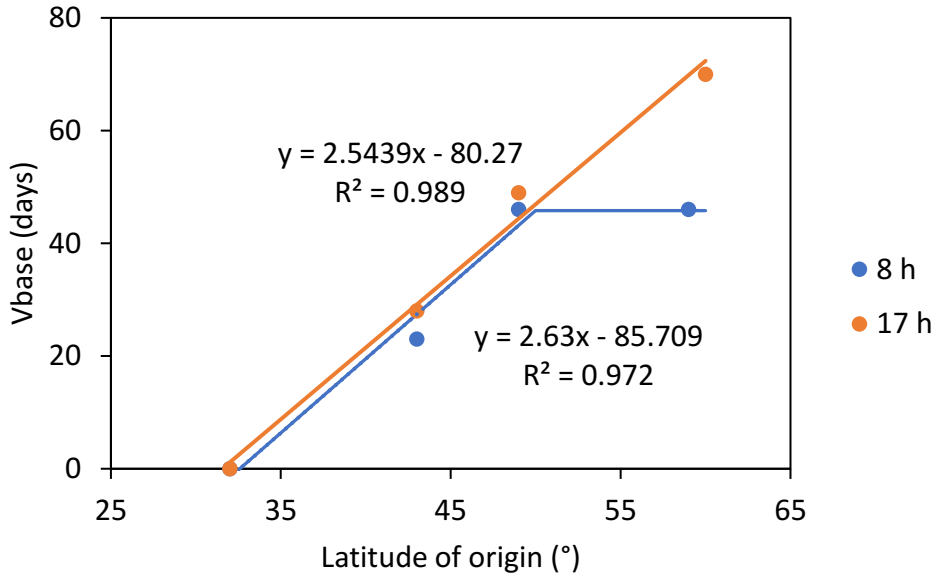


Figure 4.23. Vernalisation days to V_{base}, when 50% flowering can be expected, for four genotypes of perennial ryegrass from difference latitudes of origin when grown in controlled environment chambers when vernalisation occurred in either 8 or 17 hour photoperiod (Pp) at Lincoln University, New Zealand. 8 h Pp data derived from 4,8, and 12°C temperatures while 17 h data is derived from 4°C only data.

4.3.12 Calculations using Vernal days

Vernalisation progress can be calculated as per Equation 23

Therefore, if a maximum temperature for vernalisation is assumed to be 13°C (requires confirmation) the vernalisation effectiveness for 'Kleppe' and 'Grasslands Nui' in short Pp can be calculated as:

$$Veff = 1 \quad \text{If: } 4°C < T < 12°C$$
$$Veff = \frac{T - -4°C}{4°C - -4°C} \quad \text{If: } -4°C < T < 4°C \qquad \text{Equation 23}$$
$$Veff = \frac{13°C - T}{13°C - 12°C} \quad \text{If: } 12°C < T < 13°C$$

where T is the current temperature.

For genotypes 'Medea' and 'Grasslands Impact', which showed a linear relationship between temperature and V_{eff}, daily the vernalisation effectiveness values can be given by substituting vernalisation rate (VAI, slope of the rate of saturation regression) and VBEE (the intercept of the rate of saturation regression) from Figure 4.20.

$$Veff = \mathrm{VAI} \times \mathrm{T} + \mathrm{VBEE} \qquad T_{Vmin} \leq T \leq T_{Vopt}$$
$$Veff = \left(\mathrm{VAI} \times T_{Vopt} + \mathrm{VBEE}\right) \times \frac{(T_{Vmax} - T)}{(T_{Vmax} - T_{Vopt})} \qquad T_{Vopt} < T \leq T_{Vmax} \qquad \text{Equation 24}$$
$$Veff = 0 \qquad T_{Vmin} > T \text{ or } T > T_{Vmax}$$

Where:

Genotype	VAI	VBEE	Topt	$T_v$max	V_{base} (proportion)[1]		Rate of change/h Pp
					8 h Pp	17 h Pp	
Medea	0.0396	0.2679	18		0.000	0.000	0
Kleppe	0	0.0143	12	13	0.666	0.833	0.0186
Grasslands Nui	0	0.0124	12	13	0.666	0.875	0.0233
Grasslands Impact	0.0417	0.4889	12	13	0.416	0.680	0.0293

[1.] where a value of 1 is equal to V_{sat}

The progress toward V_{sat} is the sum of daily V_{eff}, where V_{base} and V_{sat} are genotype specific parameters.

$$V_{prog} = \sum_{day=1}^{n} V_{eff} \qquad \text{Equation 25}$$

The progress toward V_{sat} is then the sum of daily V_{eff}, and V_{base} is a genotype specific parameter dependent on Pp. V_{prog} is the sum of the daily V_{eff} values and no progress towards FI occurs until V_{base} has been reached. V_{sat} is achieved when accumulated V_{eff} reaches unity (Equation 25).

4.3.13 Upper temperatures for PI in 17 hour Pp

In 17 h Pp the upper temperature limit for vernalisation decreased from 18°C in Medea (the highest temperature tested) to 4°C in 'Kleppe' as the latitude of origin increased (Figure 4.24). 'Grasslands Nui' conformed to the relationship when a latitude of 49° was used representing a UK origin, but did not conform when a South Auckland origin (37°) was plotted. Thus, for genotypes that require chilling as part of the PI phase, a model described by; $T_{vernmax}$ = 12° -0.508/°latitude for latitudes of origin above 43° is proposed (Figure 4.24).

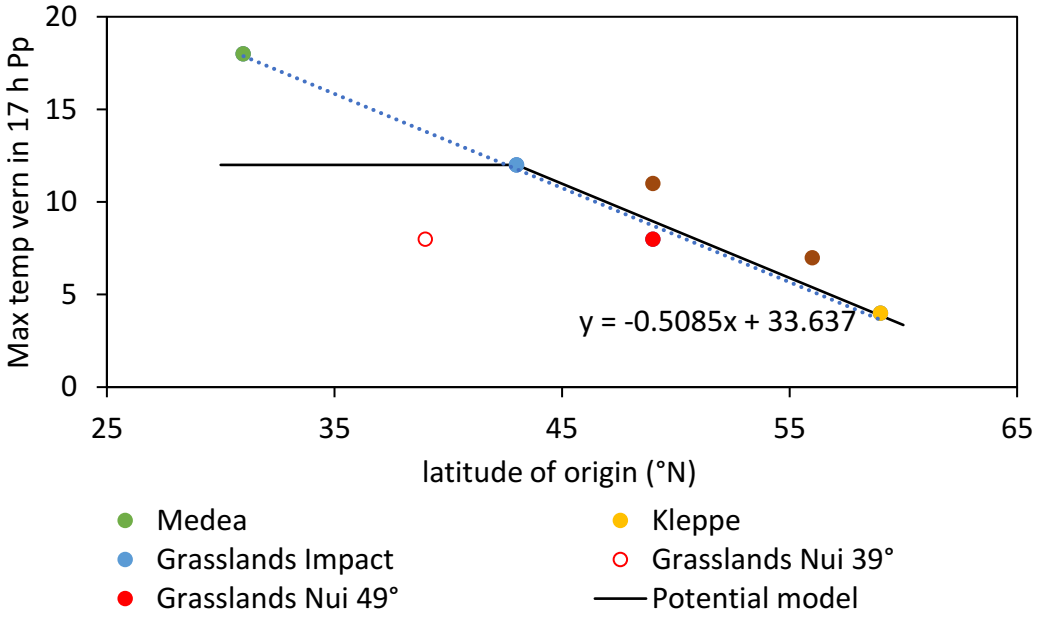


Figure 4.24. Maximum temperature for vernalisation of four perennial ryegrass genotypes when grown in 17 hour Pp inside controlled environment chambers at Lincoln University, New Zealand. Additional two genotypes plotted from (Aamlid *et al.* 2000).

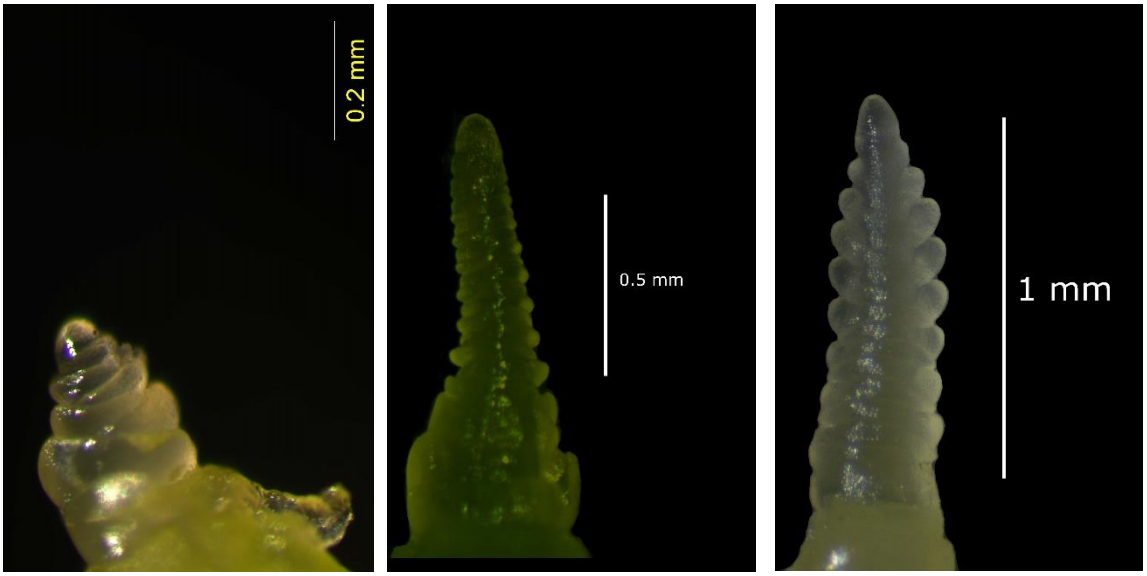
4.3.14 Modelling photoperiod influence on maximum temperature

The maximum temperature for vernalisation decreased as Pp increased from 8 to 17 h. Thus, if V_{sat} is not acquired prior to spring, a function that reduces $T_{vernmax}$ is required for modelling. This relationship was assumed to be linear and can be calculated from the maximum temperatures defined for both 8 and 17 h Pp by finding the slope and intercept of a line between two points from which Tv_{max} can be calculated from daily Pp.

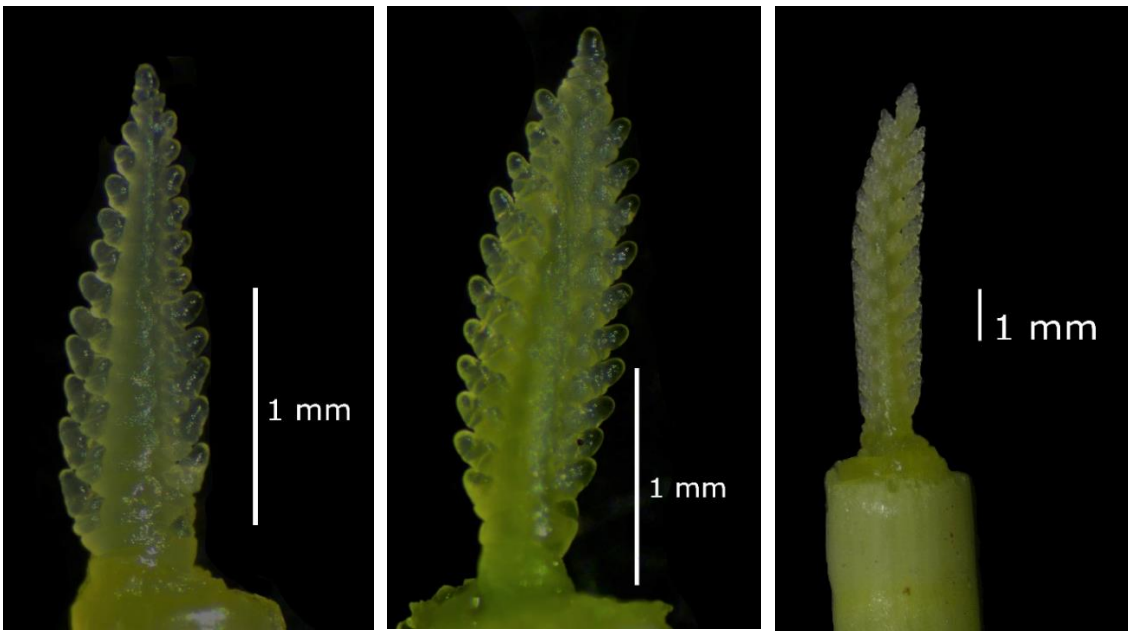
Table 4.6. Maximum temperatures for vernalisation (Tv_{max}) at two photoperiods (Pp) and the relationship required to calculate the maximum temperature at intermediate Pp of four genotypes of perennial ryegrass.

Genotype	Max temperature (°C)		Slope	Intercept
	8 h Pp	17 h Pp		
Medea	18	18	0.00	18.0
Kleppe	12	4	-0.89	19.1
Grasslands Nui	12	8	-0.44	15.56
Grasslands Impact	12	12	0.00	12

Where Tv_{max} = Slope × Pp + Intercept, for Pp between 8 and 17 hours.



Vegetative | Double ridge | Spikelet formation



Early terminal spikelet | Terminal spikelet | Past terminal spikelet

Figure 4.25. Examples of stem apices from dissections at different stages of development.

4.4 Discussion

The objective of this chapter was to quantify the vernalisation and thermal time requirements for the phenophases up to final leaf emergence of four representative perennial ryegrass genotypes. This was achieved through a series of controlled environment experiments where genotypes were exposed to four temperatures in both 8 and 17 h Pp. Based on the occurrence of genotype differences in the percentage of plants flowering, the null hypothesis was rejected.

4.4.1 Plants flowering

For flowering, all genotypes were obligate long day plants, flowering only when exposed to long Pp but they differed in their vernalisation response. 'Medea' showed no vernalisation response (Figure 4.7) while the other genotypes are obligate for vernalisation, followed by a facultative response with further exposure to vernalising conditions. The requirements for satisfying vernalisation were least for 'Grasslands Impact', increasing for 'Grasslands Nui', and greatest for 'Kleppe'. Thus, the vernalisation requirements increased as the latitude of origin increased supporting the findings of Cooper (1960) and Aamlid *et al.* (2000).

The results for 'Medea' are consistent with the cultivar 'Veyo' described by Aamlid *et al.* (2000). Together these genotypes represent a gene pool originating near the Mediterranean Sea. These genotypes appear to have similar requirements for reproductive development as spring wheat, where Pp above a base value induces flowering (Jamieson *et al.* 1995a). 'Medea' originated from Algeria (~32° N) where low temperature exposure is limited and Pp provides the major driver for FI. However, the range in Pp is small at ~4.5 h, thus the sensitivity to Pp must be strong. The 17 h Pp used in these experiments is longer than the maximum of 15.3 h experienced at 'Medea's' centre of origin (Pp calculation includes civil twilight). It is potentially possible that the longer than natural Pp used in this experiment was able to override any limitations of flowering.

'Grasslands Nui', 'Grasslands Impact' and 'Kleppe' all showed an obligate requirement for vernalisation to complete FI. All genotypes achieved flowering when vernalisation

occurred in 8 h Pp at 12°C or less, while flowering took longer, or did not occur at all, in long photoperiods. This result is similar to those presented by Aamlid *et al.* (2000) where temperatures of 9°C or less induced flowering in the genotype 'Falster' and 15°C or less in 'Baca' when the Pp was 10 h during vernalisation and that induction was slower in long Pp. Thus, perennial ryegrass expresses a typical 'short-day' vernalisation response where short Pp and low temperature promotes progression towards V_{sat}. Combined, these data support an upper limit for vernalisation of between 12 and 15°C for perennial ryegrass in short (8-10 h) Pps, potentially dependent on germplasm origin as suggested by Aamlid *et al.* (2000).

4.4.2 Temperature requirements

When vernalisation occurred in 17 h Pp, the temperature required to achieve FI reduced as latitude of origin increased. For example, 'Grasslands Impact' required 12°C or below while 'Grasslands Nui' (<8°C) and 'Kleppe' (4°C) required cooler temperatures to overcome a Pp induced restriction to achieve FI (Figure 4.7). 'Grasslands Impact' originates from coastal NW Spain (~43° latitude), 'Grasslands Nui' likely originates from English germplasm (~54° latitude) while 'Kleppe' was collected in Norway (~59° latitude). These data support Aamlid *et al.* (2000) who suggested temperatures of 7-11°C were required to achieve FI in long days for genotypes originating from Denmark (~56° latitude) and Czech Republic (~50° latitude), respectively. Aamlid *et al.* (2000) also showed similar response to 'Grasslands Nui' with the genotype 'Baca' where 50% flowering was achieved when vernalised in short days, up to 15°C. However, in continuous lighting flowering above 9°C was limited, thus long Pp inhibited progress towards flowering. These temperatures are higher than the 6°C suggested by Cooper (1960) and Evans (1960) as required for primary induction in long Pp, a result supported by the 'Kleppe' data. These data suggest that differences exist among genotypes in their ability to be vernalised under long Pp. This potentially has implications for FI from spring sowings, where both temperature and Pp are continually increasing. Thus, genotypes from lower latitudes e.g. 'Grasslands Impact' appear more likely to achieve FI from spring sowing in lengthening days and increasing temperatures compared with those from higher latitudes e.g. 'Kleppe'. Combined, these data suggest the upper limit for vernalisation in long Pp is genotype specific and related to latitude of origin (Figure 4.24). This ranged from 4-6°C

for genotypes represented by ‘Kleppe’ to 12-15°C for those represented by ‘Grasslands Impact’ and ‘Baca’. The temperature required to induce FI reduced by 0.5°C/° of latitude above 32° (Figure 4.24) when vernalisation occurred in a 17 h Pp. The implication is that there is a ‘backup’ mechanism for genotypes from northern latitudes that ensures winter is encountered before FI occurs. Thus, a genotype specific response to increased Pp reduced the effectiveness of cool temperatures required to allow FI.

Differences among genotypes occurred in the duration required at each temperature to achieve FI (Figure 4.7). In 8 h Pp, ‘Grasslands Impact’ required between two and four weeks of vernalisation, at temperatures ranging from 4–12°C while ‘Kleppe’ and ‘Grasslands Nui’ generally required six to eight weeks and ‘Medea’ did not require vernalisation to achieve flowering. The two to four week requirement of ‘Grasslands Impact’ is less than much of the published literature, while the ‘Grasslands Nui’ and ‘Kleppe’ results are similar to the 6–10 weeks of vernalisation generally reported for perennial ryegrass (Cooper 1960; Evans 1960; Aamlid *et al.* 2000). Therefore, vernalisation responses are genotype specific and range from facultative to obligate.

4.4.3 V_{base}

When vernalisation occurred in 17 h Pp, the response slowed such that an extra two weeks of exposure was required to induce flowering (V_{base}) (Figure 4.7). For example, at 8°C ‘Grasslands Nui’ required eight weeks in 17 h Pp compared with six weeks in 8 h Pp. At 4°C this trend continued where ‘Kleppe’ required 10 weeks in 17 h Pp or eight weeks in 8 h Pp. At 4°C, ‘Grasslands Nui’ and ‘Grasslands Impact’ achieved V_{base} after four weeks in both Pps supporting data from Cooper (1960) and Evans (1960) that temperatures below 6°C render perennial ryegrass insensitive to Pp. However, in each of these examples, low temperature exposure overcame the photoperiod constraint and allowed flowering to proceed below a genotype specific upper temperature limit. Therefore, genotype specific responses to temperature are required for model parameterisation in long Pp.

The duration of vernalisation required to obtain flowering (V_{base}) can also be referred to as a ‘lag phase’, where the accumulated response does not lead to FI. In most cases the

lag phase to obtain V_{base} was longer in 17 h Pp than 8 h Pp at temperatures of 8°C or above. The obtainment of V_{base} does not confer the vernalisation requirement has been saturated (V_{sat}). The response is facultative were further exposure reduces the number of main stem leaves and thus the time to final leaf emergence following exposure to long days (Figure 4.17, Figure 4.18).

4.4.3.1 Modelling V_{base}

The 'Sirius' approach (Section 2.6.2) requires the addition of V_{base}, or a lag phase of vernalisation where no flowering occurs. The V_{base} for perennial ryegrass was significantly longer, at 6 weeks or 42 VDD for 'Grasslands Nui' and 'Kleppe' (Table 4.5), than the 8 VDD required for wheat at optimum temperatures in ARCWHEAT1 (Weir *et al.* 1984). In days, wheat has a lag phase of between 14 and 49 days in 8 h Pp depending on temperature where no change is recorded in FLN, but flowering eventually occurs due to the facultative response (Brooking & Jamieson 2002). In perennial ryegrass, the lag ranged from zero days in 'Medea' to 46 days in 'Grassland Nui' and 'Kleppe'. This is supported by Aamlid *et al.* (2000) who demonstrated that 42 days (six weeks) was required to achieve flowering in two genotypes. Others required between six and nine weeks when vernalisation occurred at 6°C in 8 h Pp, within the optimum range presented here. Cooper (1956a) suggested that perennial ryegrass requires longer vernalisation than many annual species and therefore a longer lag phase is not unexpected. Thus, the lag phase is genotype specific and increased by ~2.35 days for each degree increase in latitude of origin increase above 32° (Figure 4.21, Figure 4.22, Figure 4.23). However, 46 days appears standard if V_{base} is unknown for central and northern European genotypes.

'Medea' acted like spring wheat (Brooking & Jamieson 2002) with no lag phase to achieve V_{base} but did demonstrate small reductions in FLN following transfer from 8 h Pp conditions, up to 28 days (Figure 4.18). This response was similar to the genotype 'Veyo' in the study by Aamlid *et al.* (2000) which required zero weeks vernalisation to achieve flowering. Therefore, a V_{base} of zero for this group of germplasm is appropriate. In 17 h Pp, 'Medea' produced an almost constant FLN at all temperatures which is similar to the annual species *L. temulentum* and *L. multiforum* presented by Evans (1960). This suggests FI occurred at a similar HS for all temperature treatments.

In general, a longer lag phase was present in 17 h Pp compared with 8 h Pp when temperatures were above 4°C. A result consistent with Cooper (1956a) for ryegrass and wheat (Brooking & Jamieson 2002; Brown *et al.* 2013). Thus, V_{base} increases as Pp increases at a rate dependent on genotype.

4.4.4 FLN

In 8 h Pp, there was a steady increase in FLN following continued treatment exposure, regardless of temperature, to a maximum of 16 leaves after 12 weeks in 18°C (Figure 4.17). This result follows the same trend as spring wheat data presented by Brooking and Jamieson (2002), and reanalysed by Brown *et al.* (2013), where FLN increased in relation to temperature from ~8 to 13 leaves, following 84 days (12 weeks) exposure to 11°C, 8 h Pp. For 'Grasslands Nui', 'Grasslands Impact' and 'Kleppe', FLN initially reduced and at 4°C remained constant. At 8 and 12°C, FLN reduced to a minimum at approximately 8 weeks following which FLN began to increase as the duration of treatment exposure was extended. This response was most evident in 'Grasslands Impact' where FLN reduced to match 'Medea' at eight weeks. Brown *et al.* (2013) showed a similar pattern with a reduction in FLN followed by a subsequent increase for winter wheat after approximately 60, 50, 50 and 40 days of vernalisation at 5, 8, 11 or 23°C respectively in 8 h Pp. However, the mechanisms for the increase in FLN are different. In wheat FI was commonly achieved under an 8 h Pp and subsequently the short Pp delayed the time from FI to TS. In this experiment, no FI took place for plants exposed to an 8 h Pp (Figure 4.4). This confirms an obligate requirement for long Pp following vernalisation. Thus, the increase in FLN was associated with an increase in HS during vernalisation as plants required an appropriate Pp to induce FI (Figure 4.5). No other data were found for perennial ryegrass where FLN was reported following vernalisation in short Pp, for different durations, followed by transfer to long Pp. Most authors only present data on days to heading, following transfer to long days where the duration reduces as the vernalising duration increases. Without the FLN, quantifying the influence of vernalising treatments on the duration to flowering can be confounded by temperature, where warmer temperatures increase the rate of leaf appearance. Therefore, data reported in days to heading must be treated with caution.

When grown in 17 h Pp, the FLN for 'Medea' was relatively constant at ~9. It reduced by ~0.5 HS between 0 weeks and each treatment minimum, similar to the spring wheat response presented by Brown *et al.* (2013). For 'Grasslands Nui', 'Grasslands Impact' and 'Kleppe', FLN initially reduced and flattened in treatments where FI was achieved. This result supported Silsbury (1964) who showed that the final number of main stem leaves plateaued after six weeks of vernalisation at 2°C in complete darkness, for durations up to 12 weeks. Overall the FLN of 'Medea' follows a trend consistent with that for spring wheat shown by Brooking and Jamieson (2002). The remaining genotypes, appeared obligate for vernalisation. They followed a response consistent with winter wheat in 8 h Pp. The major difference is, the perennial ryegrass genotypes did not achieve FI below a genotype specific V_{base}. However, if FLN's above 15 for wheat are ignored, then the patterns are similar (Brooking & Jamieson 2002), which highlights the obligate requirement. Similar trends were observed in the 17 h Pp data, where FLN became constant for both perennial ryegrass and winter wheat. This occurs after high FLNs are reduced in wheat, but those do not appear in perennial ryegrass due to the obligate requirement for vernalisation.

4.4.5 Leaf number post transfer

When grown under 8 h Pp and subsequently transferred to 17 h Pp, the decline in the leaf number post transfer (LN_{PT}) provides a robust method of determining when V_{sat} had occurred (Figure 4.18A,C,E,G). All treatments that achieved reproductive development, following vernalisation in 8 h Pp, conformed to produce ~4 leaves post transfer to 17 h Pp. These data are similar to the four main stem leaves produced after V_{sat} in genotype 'Grasslands Samson' when vernalised as seeds for at least six weeks by McCormick *et al.* (2014). Vernalisation occurred as seeds where V_{sat} and FLN were determined retrospectively by counting main stem leaves associated with nodes at head emergence. These data sets also align with Aamlid *et al.* (2000) who demonstrated a reduction in the number of days to heading from ~60 to 25 for five perennial ryegrass genotypes when grown in glasshouse conditions. They had a mean temperature of 18°C and 17-18 h Pp. If the phyllochron was 120°C days, then most genotypes conformed to the emergence of four leaves post transfer at the equivalent of V_{sat} (15 weeks at 6°C in 8 h Pp). This can be

calculated as 25 days x 18°C = 450°C days, 450/120 = 3.75 leaves. Thus, the minimum possible number of leaves post V_{sat} is four, when the SI requirement is saturated.

As accumulated vernalisation approached V_{sat}, a decreasing number of leaves was produced following transfer to 17 h Pp. Similarly, Evans (1960) demonstrated that temperatures of 4, 7 and 10°C for 16 weeks in short (8 h) days required a similar number of days to heading. In contrast, temperatures which produced a partial response required more days to achieve heading. Aamlid *et al.* (2000) showed no floral induction with less than six weeks vernalisation (consistent with 'Grasslands Nui' and 'Kleppe') and a reduction in time to heading as exposure continued. Since, Evans (1960) and Aamlid *et al.* (2000) did not dissect stem apices the mechanism for reductions in days to heading is unclear. However, a reduction in the LN_{PT} is a plausible explanation for the reduction in days from transfer to heading and supports the facultative response post V_{base}.

When primary induction occurred in 17 h Pp, treatments produced fewer leaves following transfer as the duration of vernalisation increased. That is, they reached FI in treatment conditions while simultaneously producing leaves prior to transfer (Figure 4.5). For example, 'Grasslands Impact' demonstrated signs of FI prior to transfer into 18°C conditions when treatment occurred at 4 and 8°C, in 17 h Pp. 'Medea' showed signs of FI on transfer at all temperatures tested in 17 h Pp (Figure 4.4) and LN_{PT} decreased (Figure 4.18). Therefore, LN_{PT} does not explain when V_{sat} occurs during vernalisation in long days, thus the lowest FLN is more appropriate.

At 4°C the duration required to achieve V_{sat} was the same in both 8 and 17 h Pp for 'Medea', 'Kleppe' and 'Grasslands Nui'. Therefore, temperatures of 4°C removed any Pp influence on the time to V_{sat}. This supports data presented by Cooper (1960) who suggested at temperatures below 6°C, perennial ryegrass plants became insensitive to Pp. For 'Grasslands Impact,' the duration to V_{sat} was four weeks shorter in 17 h Pp than 8 h Pp at 4°C, suggesting some variation among genotypes. Alternatively, at 8 and 12°C, the duration for 'Grasslands Impact' was longer in 17 h Pp, thus a temperature by Pp interaction for the treatment duration to V_{sat} occurred in this genotype (Figure 4.19). A similar reduction in treatment duration to achieve V_{sat} in 17 h Pp was shown for

'Grasslands Nui' at 8°C. However, the FLN response to calculate V_{sat} for 'Grasslands Nui' was small and may not represent a meaningful change (Figure 4.17). Surprising, little data were found in the literature that supplies the number of leaves when perennial ryegrass is vernalised in long Pp to allow direct comparison. Evans (1960) presented data for a New Zealand ryegrass that demonstrated a maximum temperature of 8°C during vernalisation to achieve 50% of plants to flower in a 16 hr Pp. This example had a fixed duration of 16 weeks, but there was no data for leaf number.

These changes suggest there are genotype differences in the duration of primary induction required to achieve both V_{base} and V_{sat}. This is particularly evident where Pp increases where corresponding decreases in temperature are required to compensate. For example, in the 17 hr Pp, 'Kleppe' did not achieve flowering at 8°C while 'Grasslands Nui' did not flower at 12°C, but 'Grasslands Impact' flowered at both 8 and 12°C. All three genotypes flowered at these temperatures under an 8 h Pp. The implication is that there are differences among genotypes in how the Pp genes affect flowering in a long Pp. Practically, this combined with a shorter duration to obtain V_{base}, suggests that 'Grasslands Impact' is more likely to flower from late winter sowing than 'Grasslands Nui' or 'Kleppe'.

4.4.5.1 Modelling V_{sat}

Combining individual controlled environment chamber experiments and converting the duration to V_{sat} (Table 4.4) into, rate of progress towards V_{sat}/day, allows the separation of temperature *per se* on vernalisation. The slope of the linear regression gives the rate of progress towards V_{sat} for each genotype (Figure 4.19). For example, 'Medea' showed the greatest response to temperature with the shortest duration requirement for V_{sat} and had a slope of 0.0014/°C. This is consistent with the 0.0012/°C presented for wheat, cultivars 'Agent' and 'Mit' by Robertson *et al.* (1996). For 'Grasslands Impact' values were in line with other wheat cultivars. The values resulting from substituting daily mean temperatures for x in Equation 25, can be summed to provide an indication of progress towards V_{sat}, similar to the *Sirius* method, where a value of 1 would provide saturation (He *et al.* 2012). However, 'Grasslands Nui' and 'Kleppe' both produced a flat response (days to V_{sat}, thus 1/days) to temperatures between 4 and 12°C, with y intercepts of

0.0143 and 0.0125, respectively. Thus, in 8 hr Pp, temperatures of between 4 and 12°C were equally effective at progressing towards V_{sat} in 'Grasslands Nui' and 'Kleppe', similar to the ARCWHEAT1 method. For 'Grasslands Impact', 12°C showed greater efficacy than either 4 or 8°C (Figure 4.20). Genetic differences among genotypes is probable as Evans (1960) showed the rate of vernalisation was quicker at 10°C compared with 4°C, consistent with the results for 'Grassland Impact'. However, Heide (1994) suggested 3°C was the lower limit in both short and long days and that effective upper limits for short and long days are missing for this important species. Aamlid *et al.* (2000) added information around the upper limits of temperature for vernalisation but only in set durations of 10 or 12 weeks, thus not adding to the effectiveness of different temperatures.

The ARCWHEAT1 modelling method (Section 2.6.1) requires the calculation of Vdd based on the effectiveness of different temperatures. Based on the time to V_{sat}, in 8 h Pp, temperatures were assigned a relative effectiveness in relation to the number of days of vernalisation required to achieve V_{sat}, where the minimum duration was the most effective temperature (Figure 4.20). Thus, Vdd can be calculated via the same methodology as ARCWHEAT1. However, the temperature response for 'Grasslands Impact' and 'Medea' does not have a plateau, meaning the calculation method required adaption to include a linear response as per Equation 24, similar to the *Sirius* approach. 'Grasslands Nui' and 'Kleppe' followed a pattern consistent with the standard ARCWHEAT1 approach, but minor adjustment of the cardinal temperatures are required, particularly at warmer temperatures (Equation 4 Figure 2.3). ARCWHEAT1 uses 10°C to set the upper limit of the optimum range, but the current data suggests this should be increased to 12°C, based on results for 'Grasslands Nui', 'Grassland Impact' and 'Kleppe'. The upper limit for a contribution to vernalisation in ARCWHEAT1 is set to 17°C, but for perennial ryegrass this is lower because no flowering occurred in temperatures of 18°C. Aamlid *et al.* (2000) suggested the upper temperatures for vernalisation of two genotypes, with similar latitudes of origin to 'Grasslands Nui' and 'Kleppe', were 15 and 11°C respectively. Thus, the mean value of 13°C appears a sensible starting point for modelling, until genotype specific values are derived. The current study supports a lower optimum of 4°C, which was the lowest temperature tested, while Heide (1994) suggested

3°C was the lower optimum. Little work exists to identify an absolute lower limit temperature for vernalisation. However, many authors have successfully induced perennial ryegrass at temperatures of 1-3°C (Cooper 1960; Silsbury 1964). Since vernalisation is a metabolic activity, it makes sense to assume progress reduces, if not stops, as temperatures approach freezing. Thus, maintaining the current -4°C is favoured as the effectiveness at 0°C was the same as extrapolation of 'Grasslands Impact', half that of the optimum (Figure 4.20), while temperatures below 0°C accumulate low levels of effective vernalisation/day.

4.4.6 Leaf appearance

A two straight line model was used to describe leaf appearance rate (phyllochron) where prior to ~8 leaves, the phyllochron was ~105°C days but then increased to ~190°C days (Figure 4.6). This response is consistent with results from Experiments 1-4 (Chapter 3, Section 3.3.4) and with wheat. Brown *et al.* (2013) used a three straight line model to describe leaf appearance rate over time. The initial phyllochron was similar to that reported for perennial ryegrass at 96 - 110°C days (Davies & Thomas 1983; Bahmani *et al.* 2000; Black *et al.* 2002) while the later section is similar to the 167°C days used by Romera *et al.* (2009). From a practical viewpoint, these relationships need to be established for field grown perennial ryegrass where leaf length may not become as large, especially when defoliation occurs.

4.4.7 Primordium productions models

Primordium production followed the expected pattern where, during vegetative growth initial primordium were produced at a constant rate of approximately 2/HS. Specifically 1.97 for reproductive (Figure 4.9, Figure 4.10, Figure 4.11, Figure 4.12) and 2.01 for vegetative phases, respectively (Figure 4.8, Table 4.3). Thus, double that of the leaf appearance rate. In vegetative plants, primordium production slowed to a rate of 1/HS at ~Haun 7 (Figure 4.8). Kemp *et al.* (1989) also reported that one primordium was produced per leaf in perennial ryegrass plants that were 18 months old. Physiologically this allows for the accumulation of several leaves yet to emerge up until HS 7. Following HS7 a constant number of primordia are maintained to become leaves or spikelets in the future. Additionally, if flowering is delayed for an infinite time, then the addition of further primordium at a faster rate would lead to unsustainable stacking on the stem apex. Thus,

a base model of primordium production described by two lines with a slope of 2/HS prior to HS 7 and subsequently a slope of 1/HS is sensible (organs at HS 7 = 17.9) until FI. This pattern of primordium production prior to FI is similar to that of wheat and oats where primordium production was constant at ~2 and 1.7/HS (Sonego 2000) respectively until an increase in the primordium production rate at FI. However, wheat flowered when approx. 20 leaves were produced and as such builds a number of primordia that could become spikelets. In contrast, perennial ryegrass may never flower unless primary induction occurs, and therefore continues to produce leaves only, with no requirement for an accumulation of primordia.

In reproductive plants, the rate of primordium production increased to ~8/HS as spikelets were differentiated and then ceased at TS. This rate of initiation is in line with the 7.11/HS reported for 'Batten' spring wheat grown under long (16 h) Pp and the fully vernalised 'Batten' winter wheat at 7.3/HS (Brooking & Jamieson 2002). These relationships have shown the number of primordium on the stem apex are linearly related to the HS and thus the events on the stem apex and leaf appearance are coordinated. Therefore, it is possible to use the current number of leaves to describe apex organ number in plants where FI has not been achieved, or in plants where FI has been achieved assuming a 17 h Pp. Further information on the Pp response during SI and ear development phases is required to confirm this relationship outside a 17 h Pp. This method allows for differences in temperature *per se* as the leaf appearance rate is related to temperature and can be described using the phyllochron.

4.4.8 FI - TS

The duration from FI to TS was constant for all genotypes and PI treatments. This was described as $TS^{HS} = 0.84 \times FI^{HS} + 3.36$ or could be estimated by $TS^{HS} = FI^{HS} + 2.24$ (Figure 4.15). There was no range in Pp between FI and TS so no Pp response could be measured. In wheat, Brooking and Jamieson (2002) showed the duration from FI to TS was prolonged by exposure to 8 h Pp in an experiment with similar vernalisation treatments. However, in a reanalysis, Brown *et al.* (2013) showed a strong relationship of 2 HS between FI^{HS} and TS^{HS} for wheat when FI occurred in 16 h Pp. Further experimentation is required in Pps of ~12 h (Heide 1994) to determine if Pp increases the duration between FI and TS.

The number of leaves to emerge following TS^{HS} was conservative among genotypes, and vernalisation treatments. This allows the final number of main stem leaves (FLN) to be calculated as FLN = $TS^{HS} \times 0.98 + 2.09$ (Figure 4.16). This relationship follows wheat where Brown *et al.* (2013) described the duration between TS^{HS} and FLN as FLN = $2.86 + 1.1 \times TS^{HS}$. However, this relationship is approx. 1 HS longer than that reported by Kemp *et al.* (1989) who suggested TS was achieved when the tip of the final leaf (final leaf) emerged in both wheat and perennial ryegrass. It appears the difference can be accounted for within the description of TS. In this study, TS was defined mathematically from the three-line equations as the HS at which primordium production ceased. This was supported and cross checked against visual records from dissections (Figure 4.25). In contrast, Kemp *et al.* (1989) presented data based on visual dissections using a more developed seed head to define TS.

4.5 Conclusions

The objective of this chapter was to quantify the vernalisation requirements of contrasting genotypes. Based on a series of treatments the following conclusions can be made:

- Certain perennial ryegrass genotypes do not require exposure to temperatures less than 18°C to achieve flowering in a 17 h Pp.
- 'Medea' had an obligate long day plant response which only required a 17 h Pp to flower.
- For short Pp during PI, all genotypes were vernalised (reach V_{base}) at temperatures of 12°C or less, where each genotype required a specific duration to achieve V_{base} and V_{sat}.
- When long Pp occurred during vernalisation, cooler temperatures were required to reach V_{base}. Generally, the temperatures required followed the latitude of origin where 'Grasslands Impact' (~42°) achieved 50% of plants flowering at 12°C, after 12 weeks, while Kleppe (~60°) required 10 weeks at 4°C.
- Perennial ryegrass is obligate for long Pp following vernalisation. When vernalisation occurred in 8 h Pp, primordium production continued at the vegetative rate until plants were transferred into the 17 h Pp.
- Following V_{base}, vernalisation was a facultative process where the number of leaves produced post transfer to 17 h Pp reduced, from ~8, to a minimum of 4 when V_{sat} was achieved.
- For all the genotypes tested, short days alone did not replace the requirement for cold temperatures during the PI phase.

5 SOWING DATE TRIAL

Objective 3.

- To quantify the timing of phenophases from emergence to flowering of four perennial ryegrass genotypes grown under field conditions with natural changes in photoperiod.

Null hypothesis:

- All genotypes will reach the stages of development at the same time and demonstrate the same Pp response.

5.1 Introduction

In this chapter the vernalisation (primary induction, PI) and photoperiod (secondary induction and ear formation) requirements of four contrasting genotypes of perennial ryegrass under field conditions is investigated. Genotypes 'Medea', 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact' were used because controlled environment results in Chapter 3 and Chapter 4 showed that they have different vernalisation responses. This chapter relates to Objective 3 (Section 1); to quantify the timing of phenophases from emergence to flowering of four perennial ryegrass genotypes grown under field conditions with natural changes in photoperiod. The attainment of FI following exposure to cool temperatures was identified as the main mechanism required to determine the time of flowering in three of the four genotypes (Chapter 4). It is expected that the equations from Chapter 4 can be used to estimate when genotypes may achieve V_{base} and subsequently V_{sat}, following which the SI requirements, driven by photoperiod, will determine the timing of FI (Heide 1994). It is expected that following attainment of V_{sat}, time to final leaf appearance will be determined by the Pp response influencing the HS duration from FI-TS. Chapter 4 showed that SI is obligate for long Pp. The mechanism of this response is unclear in the literature and could potentially range from; 1) a base Pp (Pp_{base}) required to induce FI with constant time to final leaf (FL), 2) a decrease in the time between FI and TS as Pp increases towards a saturating length (Pp_{sat}) with constant HS duration from TS-FL, or 3) a combination of both.

Combining data from Chapter 3 and Chapter 4 for genotypes 'Kangaroo Valley' and 'Medea' (Figure 5.1) allows the adjustment of Equation 15 and forms a starting point for this investigation. A minimum leaf number (LN_{min}) of 8 was identified for 'Kangaroo Valley' in Chapter 3, thus LN_{min} = 8 while a breakpoint at 17 h appears appropriate (Figure 5.1), thus the saturating photoperiod (Pp_{sat}) is set at 17 as per Equation 26.

$$FLN = \begin{cases} LN_{min} + B\ (Pp_{sat} - Pp) & Pp < Pp_{sat} \\ LN_{min} & Pp \geq Pp_{sat} \end{cases}$$ Equation 26

Where: LN_{min} is the minimum possible leaf number = 8
B is the slope of the Pp response = 1.29
Pp_{sat} is the saturating Pp = 17
Pp is the current Pp

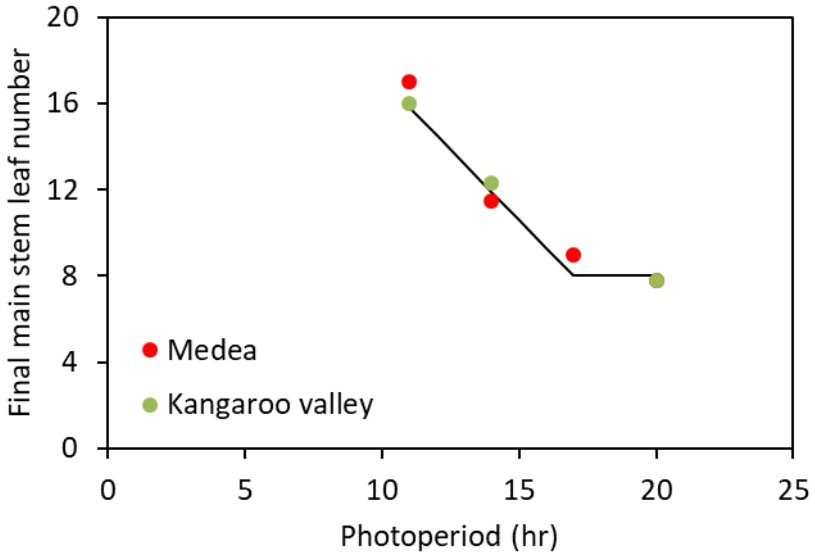


Figure 5.1. Potential model for estimating the number of main stem leaves of perennial ryegrass using data from Chapter 3 and Chapter 4. The saturating photoperiod is presented as 17 hours, when the minimum leaf number is eight and the slope of the Pp regression to that point is -1.29.

After determining the final number of main stem leaves (FLN), the timing of FL emergence can be estimated by multiplying the number of leaves by the phyllochron to determine a Tt target.

The timing of FI and TS will be determined using the same methods as in Chapter 4. This involves dissection of the apex, removing the leaves and counting the number of primordium present. Accumulated organ number (primordium and total leaf number) is then plotted against the Haun Stage (HS) (Haun 1973) to show changes in the rate of primordium production where the inflection points are FI and TS (Figure 5.2).

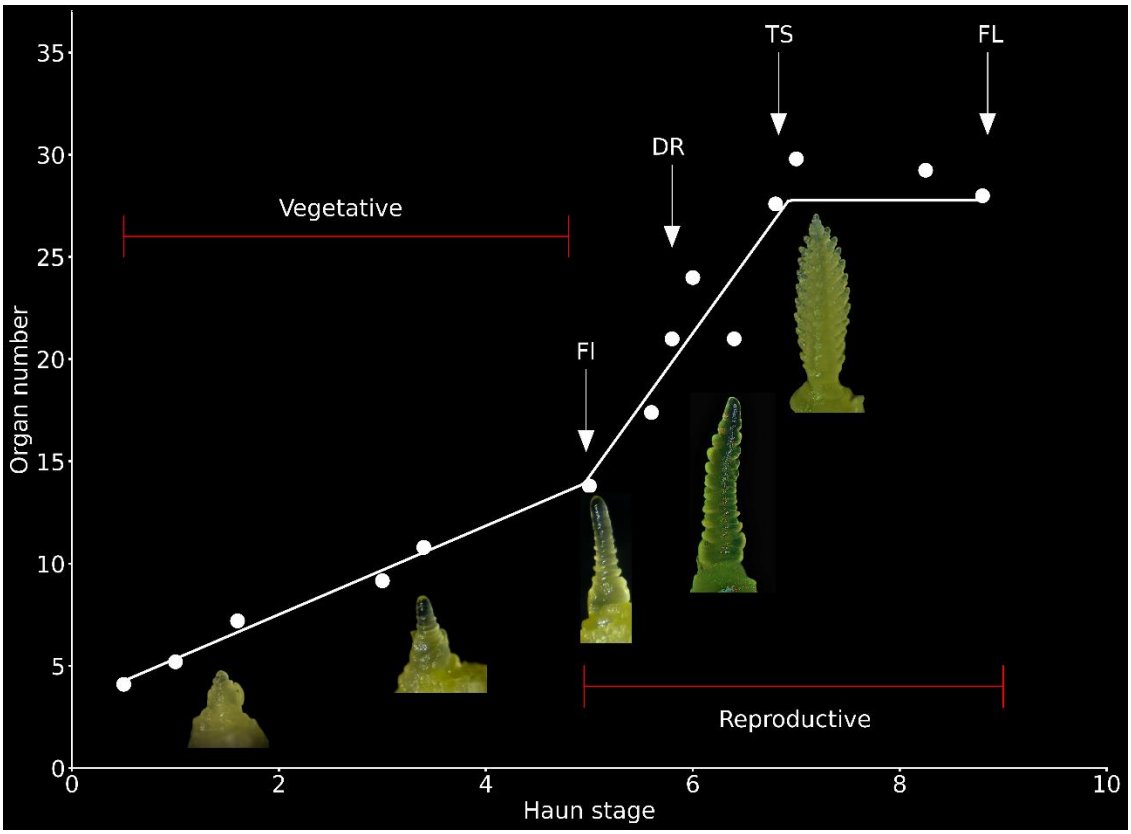


Figure 5.2. Example of the expected model from 'Medea', grown in 12°C, 8h Pp for 2 weeks followed by 18°C 17 h Pp as described in Chapter 4 to determine floral initiation (FI) and terminal spikelet (TS) relative to Haun Stage (number of main stem leaves produced) with examples of apical development. Double ridge (DR) and final leaf (FL) plotted for reference.

The time of FI and TS are expected to be different for both the genotypes and sowing dates used to ensure different Pps at the time when these developmental stages occur. This allows for Pp comparisons within and among genotypes. Haun stage assessment can be substituted for the Pp at any given time to investigate the Pp influence on FI, TS and FLN. The HS duration between FI and TS allows direct comparisons among the Pps experienced. This is because using leaf appearance rate removes the influence of temperature differences among treatments. This chapter introduces the concept that

perennial ryegrass has a juvenile phase for the perception of Pp where 4-5 HS must be achieved before FI can occur.

During this chapter three modelling methods are investigated. The first is a stepwise method where:

1. FI^{HS} is predicted from the vernalisation model and current Pp,
2. TS^{HS} is predicted from FI^{HS} and Pp,
3. FLN is predicted from TS^{HS},
4. Final leaf emergence is predicted from FLN and phyllochron,
5. Flowering date is calculated from final leaf emergence date and a Tt target.

The second approach utilises step 1 to predict the timing of FI^{HS}. Subsequently, the number of leaves to emerge post FI^{HS} is estimated based on the Pp at FI^{HS}, effectively combining stages 2 and 3 above. Leaf number multiplied by the phyllochron gives a Tt target at which final leaf appearance occurs. Finally, step 5 is used to calculate flowering date.

The third approach calibrated the Pp response within the ARCWHEAT framework and modified the accumulation of Tt based on daily Pp.

The analysis in this chapter uses the time and duration of developmental events measured in the field to build equations to estimate the time from FI to final leaf emergence which can then be used for modelling purposes and thus fulfil Objective 3. Subsequently, the duration from final leaf emergence until flowering will be analysed and equations developed to allow prediction of flowering date.

5.2 Materials and Methods

This experiment was set up on 'Kowhai Farm', located at 1321 Springs Road, Lincoln, Canterbury, New Zealand (43°64'S, 172°47'E). The soil type is a 'Wakanui silt loam' (Cox 1978) and belongs to the 'Pallic' soil order of the New Zealand soil classification system. The Wakanui silt loam has an available water holding capacity of ~170 mm in the 0-100 cm layer (Lilburne *et al.* 2012). The previous cropping history was, faba beans (*Vicia faba* L.) preceded by wheat (*Triticum avestivum* L). The field was irrigated with 60 mm of water before it was ploughed, 'Maxi-tilled' and rolled using a 'Cambridge roller'. A soil sample consisting of 150 mm deep cores was collected from the experimental site in late April and analysed by Hill Laboratories, Hamilton, New Zealand (Table 5.1).

Table 5.1. Soil test results for the experimental site, collected in late April 2019 to 150 mm depth, Kowhai Farm, Lincoln, New Zealand.

pH	Olsen P (mg/L)	Potassium (me/100g)	Calcium (me/100g)	Magnesium (me/100g)	Sulphate sulphur (mg/kg)	Mineral N (kg N/ha)
5.9	14	0.41	9.8	0.94	16	68
MAF units:		8	11	19		
Recommended minimum values[1]						
5.8	15	6	-	10	6	

[1] From Nicholls *et al.* (2009)

The genotypes selected for sowing were 'Medea', 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact'. Seed of 'Medea' and 'Kleppe' was collected from at least 30 plants that flowered at the same time in experiments from Chapter 4. At head emergence, plants were transferred in to a glasshouse that contained no other ryegrass plants and allowed to openly cross pollinate. Seed was hand harvested approximately four weeks after flowering, threshed by hand and cleaned using a seed blower. Seed of 'Grasslands Nui' and 'Grasslands Impact' was from the same seed line as that used in Chapter 4.

The experiment was hand sown as a split-plot design, with five sowing dates as main plots and four genotypes as sub plots, with three replicates. The sowing dates were 1/03/2019, 17/04/2019, 14/06/2019, 30/08/2019 and 4/10/2019. Sowing dates were selected to ensure seedling emergence occurred at different increasing and decreasing photoperiods (Table 5.2).

Table 5.2. Sowing dates and associated photoperiod at Kowhai Farm (43.6°S), Lincoln, New Zealand used to investigate the timing of floral initiation and date of final leaf appearance of four perennial ryegrass genotypes.

Treatment	Sowing date	Photoperiod (hr)	Directional change
1	1/03/2019	14.27	Shortening
2	17/04/2019	11.78	Shortening
3	14/06/2019	10.06	Neutral
4	30/08/2019	11.96	Lengthening
5	4/10/2019	13.75	Lengthening

At each sowing, ~200 seeds/m^2 were hand spread and raked in on a 1 x 5 m plot giving potentially 1000 plants in each plot.

Soil temperature was measured using a Hobo® U12 Outdoor/Industrial data logger equipped with four TMCx-HD temperature sensors each placed at 3 cm soil depth to represent soil temperature at seed and subsequently stem apex depth. Temperature recordings were taken hourly and converted into thermal time (Tt, °C days) by dividing each recording by 24. Tt was calculated via linear relationships between the base (T_b), optimum (T_{opt}) and maximum (T_{max}) temperatures. Between T_b and T_{opt}, Tt accumulated relative to the hourly temperature recorded (if T_b was above 0°C), Tt at T_{opt} was; T_{opt} minus T_b). Above T_{opt}, Tt accumulation was reduced as temperature increased, such that Tt reached zero at T_{max}. Unless otherwise stated, T_b was equal to 0°C where Tt calculations were restricted if the recorded temperature was equal or less than T_b where Tt for that recording became zero. Daily values were found as the sum of each 24-hour period.

Air temperature and humidity were measured at 1.5 m height using a Campbell Scientific CS215 sensor. Temperature within the canopy was measured 10 cm above soil level, to represent temperature at the apex during stem extension, using a Campbell Scientific 107 temperature probe housed inside a PVC radiation shield. Measurements were made at 15 minute intervals and Tt calculated as above. Rainfall and irrigation were recoded using a Davies 6463M tipping bucket rain gauge recording in 0.2 mm increments. A Campbell Scientific CR300 data logger collected data at 15 minute intervals.

Table 5.3. Monthly mean or totals of climate parameters recorded at the trial site near Lincoln, New Zealand between March 2019 and 20th March 2020. Air temperature measured at 1.5 m above ground level.

Year	Month	Mean temperature (°C)				Rainfall (mm)	Irrigation (mm)
		Air Max	Air Min	Air Mean	Soil 3 cm		
2019	3	21.8	11.7	16.2	17.6	18	-
	4	15.7	6.3	10.9	11.5	64	-
	5	16.5	5.2	10.8	9.1	19	-
	6	11.9	1.8	6.5	4.8	50	-
	7	13.2	4.3	8.6	6.8	73	-
	8	13.5	2.0	7.6	6.2	46	-
	9	14.7	4.0	9.4	8.8	31	-
	10	15.8	5.4	10.7	11.5	52	22
	11	21.2	9.7	15.4	15.7	55	69
	12	20.8	9.9	15.8	18.6	38	74
2020	1	24.0	11.7	17.9	20.3	7	116
	2	23.5	9.6	16.7	19.4	18	50
	3	22.1	12.5	16.7	16.7	4	-

5.2.1.1 Agronomic inputs

Four days prior to the initial sowing date, the experimental site was sprayed with 4 l/ha Roundup 360 (active ingredient (a.i.) 360 g/L glyphosate). In addition, prior to SD4, all remaining plots were sprayed with 5 L/ha Buster® (a.i. 200 g/L glufosinate). In crop weed control was achieved by the use of Trimec® (a.i 600 g/L mecoprop, 150 g/L MCPA and 18.7 g/L dicamba), Relay® Super S (a.i. 680 g/L 2,4-D) and Image® (a.i. 120 g/L bromoxynil, 120 g/L ioxynil and 360 g/L mecoprop-p) at different times based on sowing date (Table 5.4). Additional weed control was achieved by hand grubbing as required.

Disease control, predominantly crown (*Puccinnia coronata*) and stem rust (*Puccinnia graminis*), was achieved via a preventative fungicide program starting at Zadok's GS 31/32 based on 400 ml/ha Proline® (a.i. 250 g/l prothioconazole) and 600 ml/ha Seguris Flexi® (a.i. 125 g/l isopyrazam).

Table 5.4. Herbicide and fungicide inputs applied to four perennial ryegrass genotypes sown on five dates near Lincoln, New Zealand in the 2019/20 growing season.

Date	Type	Product and rate	Sowing date and genotypes
25/03/2019	Herbicide	4 L/ha Roundup® 360	All plots
18/04/2019	Herbicide	3.5 L/ha Trimec® and 1.25 L/ha Relay® Super S	All plots
28/05/2019	Herbicide	3.5 L/ha Image®	All plots
23/08/2019	Herbicide	5 L/ha Buster®	SD4 and 5, all genotypes
26/09/2019	Herbicide	4 L/ha Trimec®	All plots
07/11/2019	Fungicide	0.4 L/ha Proline® and 0.6 L/ha Seguris Flexi®	SD1 and 2, all genotypes
10/11/2019	Herbicide	4 L/ha Trimec®	SD4 and 5, all genotypes
30/11/2019	Fungicide	0.4 L /ha Proline® and 0.6 L /ha Seguris Flexi®	SD1 and 2, all genotypes
17/12/2019	Fungicide	0.4 L /ha Proline® and 0.6 L /ha Seguris Flexi®	SD1-4, all genotypes, SD5 for Medea
13/01/2020	Fungicide	0.4 L /ha Proline® and 0.6 L l/ha Seguris Flexi®	SD2-5, all genotypes, SD1 for Kleppe
25/01/2020	Fungicide	0.4 L /ha Proline® and 0.6 L /ha Seguris Flexi®	Medea SD4 and 5

In late winter the soil mineral N, sum of ammonium and nitrate, tested at 40 kg N/ha, with a potentially available nitrogen of 184 kg N/ha in the 0-30 cm soil profile. Perennial ryegrass needs approximately 175 kg N/ha to produce high seed yields (FAR 2013) and therefore no N was applied.

Spring and summer irrigation were applied to supplement rainfall and ensure water stress was avoided, overall 330 mm of irrigation was applied in split applications between 30 October and 25th February. Potential soil moisture deficit (PSMD) was calculated using daily potential evapotranspiration (PET) values, calculated via the Priestley-Taylor equation (Priestley & Taylor 1972), from the 'Broadfields' (Lincoln) weather station operated by NIWA (NIWA code: 17603) and located 1.5 km from the experimental site. Calculation of PSMD ceased for each SD at flowering of the latest flowering genotype. The soil was considered at 'field capacity' on 1 June 2019 following 62 mm of rainfall, where the PSMD was 0 mm, assuming full PET usage from 16 Feb 2019 when 60 mm irrigation was applied prior to cultivation. In practice, none of the plots were using the full PET values prior to 1 June 2019 as treatments took time to achieved full ground cover following sowing, thus this estimate is conservative. The lower limit PSMD target was 85

mm or 50% of the total available water holding capacity. For SD1-3, PSMD was calculated from 1 June 2019, SD4 and 5 were calculated from emergence assuming the soil was at field capacity. For SD1-3, PSMD exceeded -85 mm for five days (8-9/12/2019 and 27-29/12/2019) with a maximum potential soil moisture deficit (MPSMD) of 93 mm (Figure 5.3). However, most treatments did not reach full ground cover and/or maintain full ground cover due to constant plant removal for assessments, thus the PSMD is likely to be overestimated, once soil evaporation ceases.

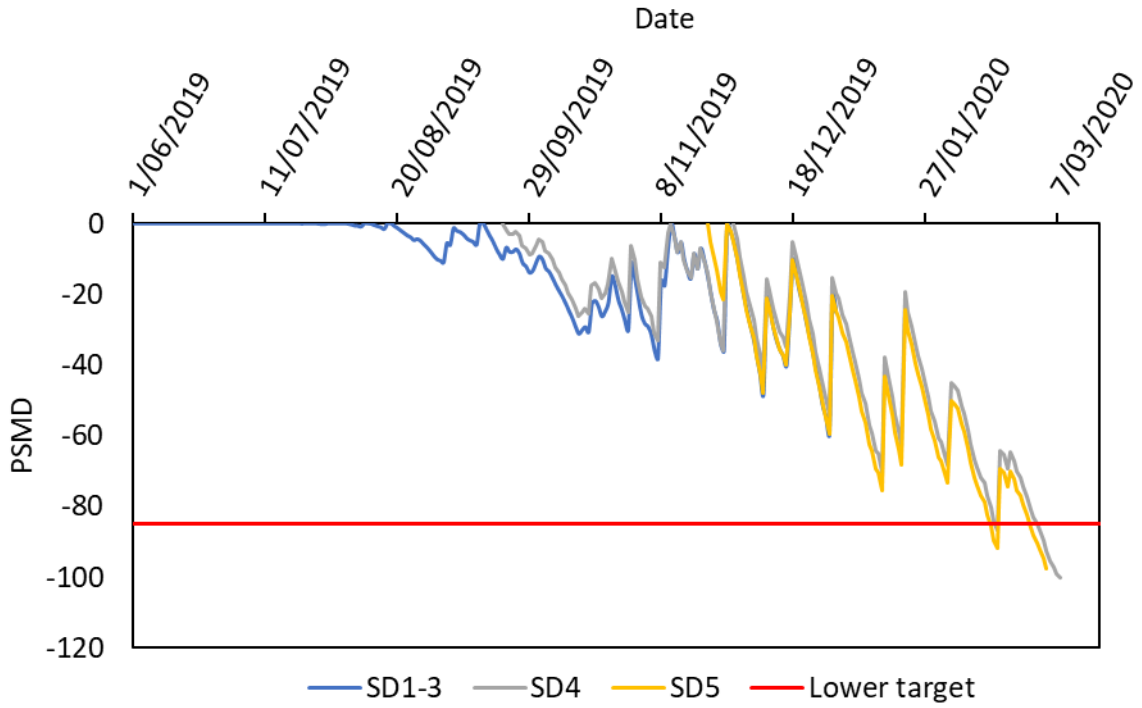


Figure 5.3. Potential soil moisture deficit (PSMD) from 'field capacity' until flowering of perennial ryegrass sown on five sowing dates (SD) at Lincoln, New Zealand in the 2019/20 growing season.

5.2.1.2 Assessments

Emergence was assessed by marking two 0.25 m^2 areas per plot following sowing and counting the number of seedlings present at 3-7 day intervals, depending on soil temperature. Assessments stopped when three consecutive assessments were the same.

Eight plants were randomly selected within a 50 × 50 cm area and plants were identified with a bamboo stake when they had three leaves. On the marked plants, the main stem

was tagged using coloured electrical wire above the first true leaf. Additional tags were placed at approximately 2-3 leaf intervals to track the main stem and leaf emergence. At each assessment the length proportion of emerging leaf was estimated relative to the youngest fully emerged leaf. These assessments were combined to give the current Haun Stage used for analysis.

At approximately weekly intervals, five plants were extracted from each plot and the leaves removed to expose the stem apex. For each plant the Zadok's growth stage, number of emerged leaves (up until ~6), length of last emerged leaf and emerging leaf, number of leaves yet to emerge and the number of primordium were counted. Observations of apex dome elongation, double ridge and terminal spikelet were recorded.

Following TS, dissections stopped but leaf counts continued until final leaf emergence (GS39). Following GS39, growth stages were assessed using the Zadoks scale (Zadoks *et al.* 1974) at weekly intervals on all tagged tillers.

5.2.1.3 Data analysis.

Time to 50% emergence was calculated by converting the plants counted at each assessment into percentage of the final count. Logistic curves were fitted to each individual plot data set using least squares regression where the start point was constrained close to zero and the maximum close to 100 (Equation 17). The inflection point described time to 50% emergence. Subsequently, curve parameters were subjected to analysis of variance (ANOVA) using Genstat® 19 (VSN 2019).

Data were collated in MS excel and interrogated utilising the packages inside the Python™ programming language. Linear regression (Equation 18) used the "olm" (Ordinary Least Squares) method in the "statsmodels" package of Python™ (Seabold & Perktold 2010). Logistic (Equation 17) and three straight lines models (Equation 21) were fitted to organ number by HS for data where treatments achieved more than 50% of plants flowering using the 'curve_fit' method inside the 'SciPy' package of Python™. For treatments that did not achieve 50% of plants flowering, data of organ number and HS were described using two straight lines fitted using the 'curve_fit' method (Equation 22). Phyllochron was

calculated by first fitting a linear regression of the accumulated number of fully emerged leaves against accumulated thermal time for each plot. The phyllochron was subsequently estimated as one divided by the slope of the linear regression equation (Equation 19).

Where data were generated for each plot, Genstat® 19 was used to complete analysis of variance (ANOVA) and to generate standard errors. Mean separation was completed by Fisher's LSD (Saville 2015). In text, the treatment means are presented as the mean ± the 'standard error of the mean' (SEM). Linear regression analysis that used treatment means was completed inside Python where standard errors, P values and R^2 data are presented as generated from the "statsmodels" package. For user defined models e.g. two and three line regressions, statistical parameters were produced from the 'curve_fit' method in the 'SciPy' package and R^2 values calculated manually (Pauli Virtanen 2020).

Where applicable, to account for the lack of flowering from SD4 and 5 for the genotypes 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact', assessments were analysed using two separate ANOVAs. First, for 'Medea' only because it achieved flowering from all sowing dates. Secondly, for all genotypes for SD1-3 to investigate genotype by sowing date interactions.

Photoperiod was calculated using standard astronomical equations in a three step process for each day of the year. Photoperiod includes the civil twilight definition from when the sun is 6° below the horizon (Equation 27) (Weir *et al.* 1984).

$$\text{coshra} = \frac{(\sin(\text{deg2rad}(\text{sun\_angle})) - (\sin(\text{lat\_RN}) \times \sin(\text{declination})))}{(\cos(\text{lat\_RN}) \times \cos(\text{declination}))}$$

$$\text{hrangl} = \cos(\text{coshra})$$

$$\text{Pp} = \text{hrangl} \times (24/(\pi \times 2)) \times 2 \qquad \text{Equation 27}$$

Where
- sun_angle = -6
- lat_RN = deg2rad(lat)
  - deg2rad = lat×π/180
    - lat = Latitude of interest = -43.6
    - π = pi = 3.14159
  - Declination = (23.4511×(2×π)/360)×sin(deg2rad(doy-82.25))
    - doy = Day of year

5.2.2 Date of Vsat.

Vernalisation progress was calculated for each genotype by sowing date combination by producing a user defined function in Python™ based on the data from Section 4. The soil temperature data collected at 3 cm depth were used to represent the apex temperature.

Table 5.5. Date when saturation of the vernalisation function occurred in four perennial ryegrass genotypes sown on five dates near Lincoln, New Zealand.

Genotype	Sowing date (SD)		Date of vernalisation saturation
Medea	1	1/03/2019	18/04/2019
	2	17/04/2019	29/05/2019
	3	14/06/2019	8/08/2019
	4	30/08/2019	13/10/2019
	5	4 /10/2019	13/11/2019
Kleppe	1	1/03/2019	12/07/2019
	2	17/04/2019	19/07/2019
	3	14/06/2019	30/08/2019
	4	30/08/2019	NA[1]
	5	4 /10/2019	NA
Nui	1	1/03/2019	12/07/2019
	2	17/04/2019	19/07/2019
	3	14/06/2019	30/08/2019
	4	30/08/2019	NA
	5	4 /10/2019	NA
Impact	1	1/03/2019	28/06/2019
	2	17/04/2019	12/07/2019
	3	14/06/2019	4/09/2019
	4	30/08/2019	NA
	5	4 /10/2019	NA

[1] NA = not achieved.

5.3 Results

5.3.1 Plant emergence

Time to 50% emergence was shorter ($P<0.001$) at 10.2 days (±0.68) for Sowing Date (SD) 1, when expressed in days after sowing (DAS), than all other sowing dates (Figure 5.4, Appendix 3). SD2 and SD5 were not different while SD3 and SD4 were slower. There was no genotype effect or genotype by sowing date interaction (i.e. $P>0.05$). SD1 produced a larger ($P<0.05$) curve shape parameter while all others were not different (Appendix 4).

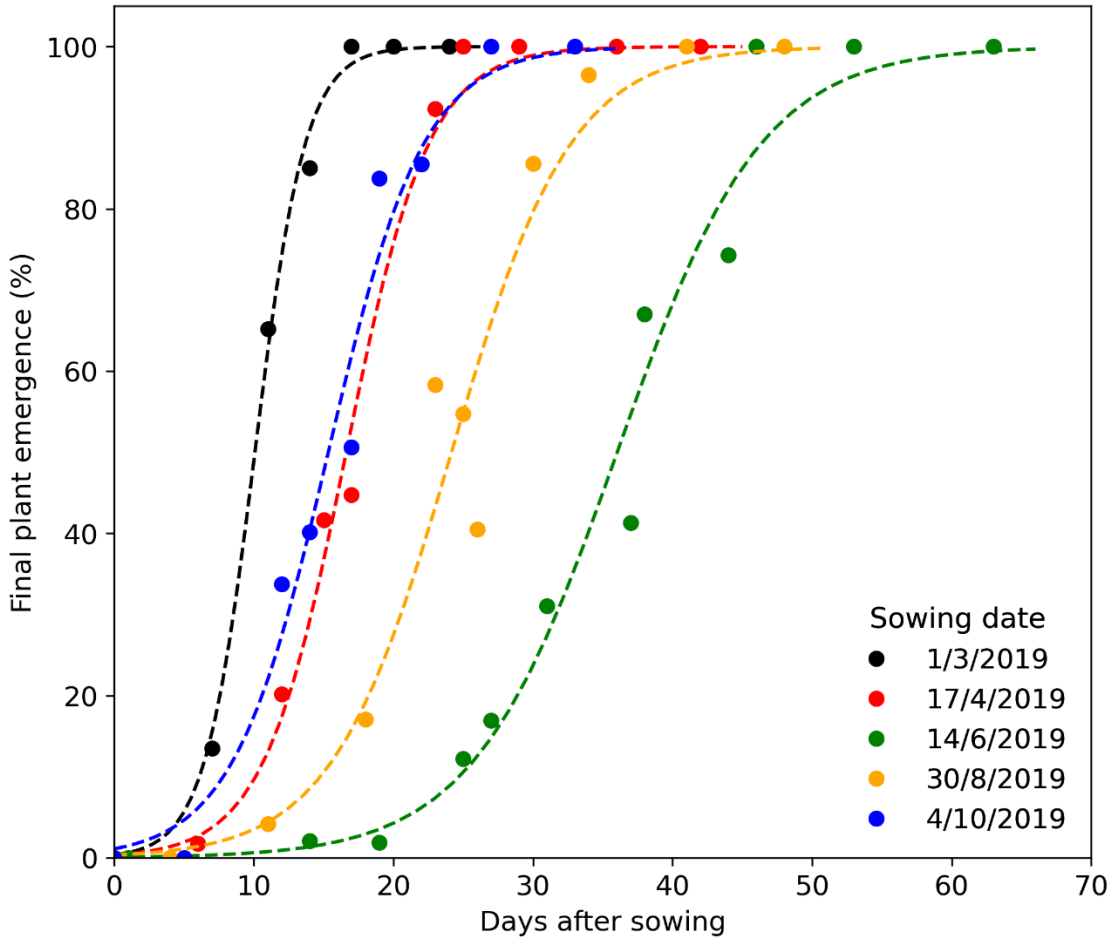


Figure 5.4. Accumulated percentage field emergence of four perennial ryegrass sown on five dates, mean of four genotypes, grown near Lincoln, Canterbury, New Zealand in the 2019/20 growing season.

Sowing date	Fitted curve parameters
1. 1/03/2019	y = 100/1+exp(-0.553(±0.047)×(***x***-10.1 (±0.178))), R^2=0.99.
2. 17/04/2019	y = 100/1+exp(-0.338(±0.039)×(***x***-16.6 (±0.335))), R^2=0.99.
3. 14/06/2019	y = 100/1+exp(-0.193(±0.027)×(***x***-36.0 (±0.777))), R^2=0.97.
4. 30/08/2019	y = 100/1+exp(-0.234(±0.053)×(***x***-24.2 (±0.836))), R^2=0.95.
5. 4/10/2019	y = 100/1+exp(-0.292(±0.045)×(***x***-13.3 (±0.493))), R^2=0.98.

When expressed in thermal time, calculated using 3 cm soil temperature and a T_b of 0°C, all sowing dates and genotypes achieved 50% emergence at 188 (±10.3)°C days with the same curve parameters (Figure 5.5, Appendix 5, Appendix 6).

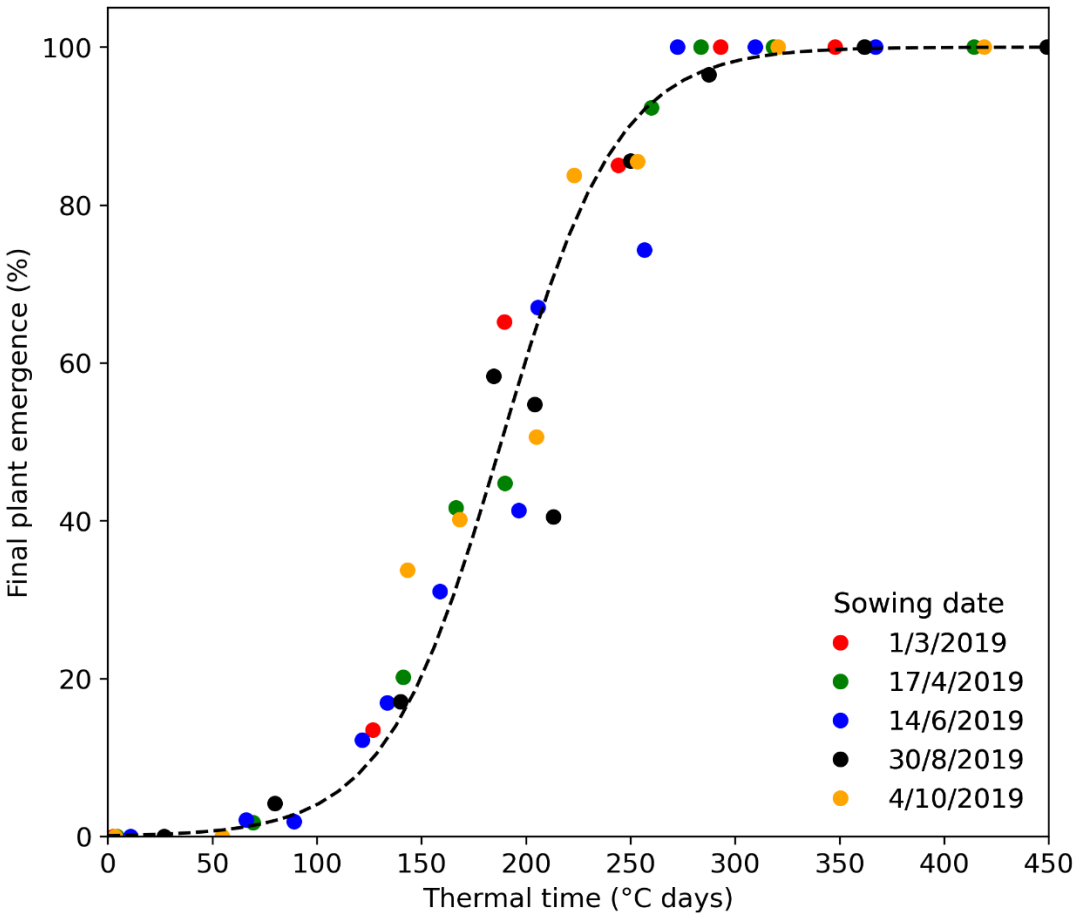


Figure 5.5. Accumulated field emergence percentage of perennial ryegrass, mean of four genotypes and five sowing dates, expressed over accumulated thermal time from sowing, T_b=0°C, sown on five dates near Lincoln, Canterbury, New Zealand. y = 100/1+exp(-0.0359(±0.0092)×(x-188 (±10.3))), R^2=0.97.

When using air temperature to calculate Tt, time to 50% emergence was affected (P<0.05) by sowing date. SD1 was faster to 50% emergence at 170°C days (± 8.8) and SD3 was slowest at 259°C days. SD2 (194), SD4 (207) and SD5 (179) were not different ($LSD_{0.05}$ = 20°C days). The overall mean was 202°C days.

5.3.2 Percentage of main stems flowering

For the percentage of plants flowering there was a genotype by sowing date interaction (P<0.001) (Appendix 9). All genotypes reached ~100% of plants flowering when sown in decreasing or day neutral photoperiods but the flowering percentage reduced the two sowing dates in spring into increasing photoperiods (Figure 5.6).

For ‘Medea’, more than 50% of plants flowered from all sowing dates, with 100% of plants flowering from autumn and mid-winter sowing (SD1-SD3) but this reduced to between 65 and 80% for spring sowings (Figure 5.6A). For ‘Kleppe’, all plants flowered when sown before the shortest day, but no plants flowered from SD4 or SD5 in an increasing photoperiod (Figure 5.6B). ‘Grasslands Nui’ and ‘Grasslands Impact’ were intermediate of ‘Medea’ and ‘Kleppe’ (Figure 5.6C,D).

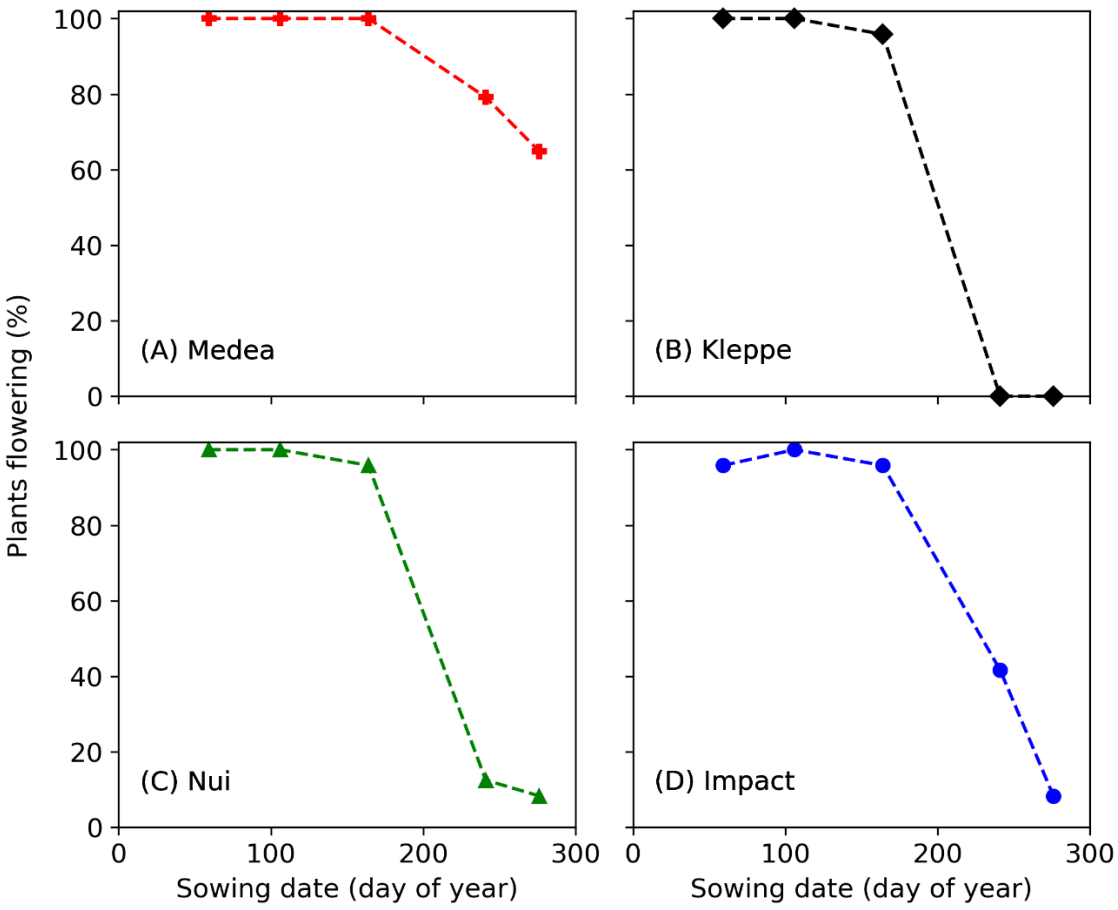


Figure 5.6. Plants flowering (%) in the summer following planting of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

5.3.3 Phyllochron

Phyllochron was calculated using 3 cm soil temperature until FI and 10 cm canopy temperature thereafter. This showed a sowing date by genotype interaction ($P<0.05$) where phyllochron increases when reproductive growth was delayed in the increasing photoperiods of SD4 and SD5 (Table 5.6). The overall grand mean was 124°C ± 4.45°C days.

Table 5.6. Phyllochron of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotypes				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	116	132	119	124	123
2. 17/04/2019	110	119	112	117	115
3. 14/06/2019	114	127	111	113	116
4. 30/08/2019	125	139	140	147	138
5. 4/10/2019	137	127	130	127	130
Genotype mean	120	129	122	126	124

Parameter	P value	SEM	$LSD_{0.05}$
Sowing date	<0.001	2.13	4.91
Genotype	0.001	2.02	4.13
Sowing date * genotype	0.001	4.45	9.02
Means within a sowing date		4.52	9.22

When the accumulated number of leaves was plotted as genotype by sowing date means, utilising a three straight line model, the phyllochron was 114°C days between emergence and HS 9.4 and 153°C thereafter (Figure 5.7), with an additional break at emergence (188°C days). Thus, HS is zero until emergence (188°C days), with a breakpoint at HS 9.5 or 1265°C days. The slope of Line 1 is 0.0088 and the slope of Line 2 is 0.0065, $R^2 = 0.96$.

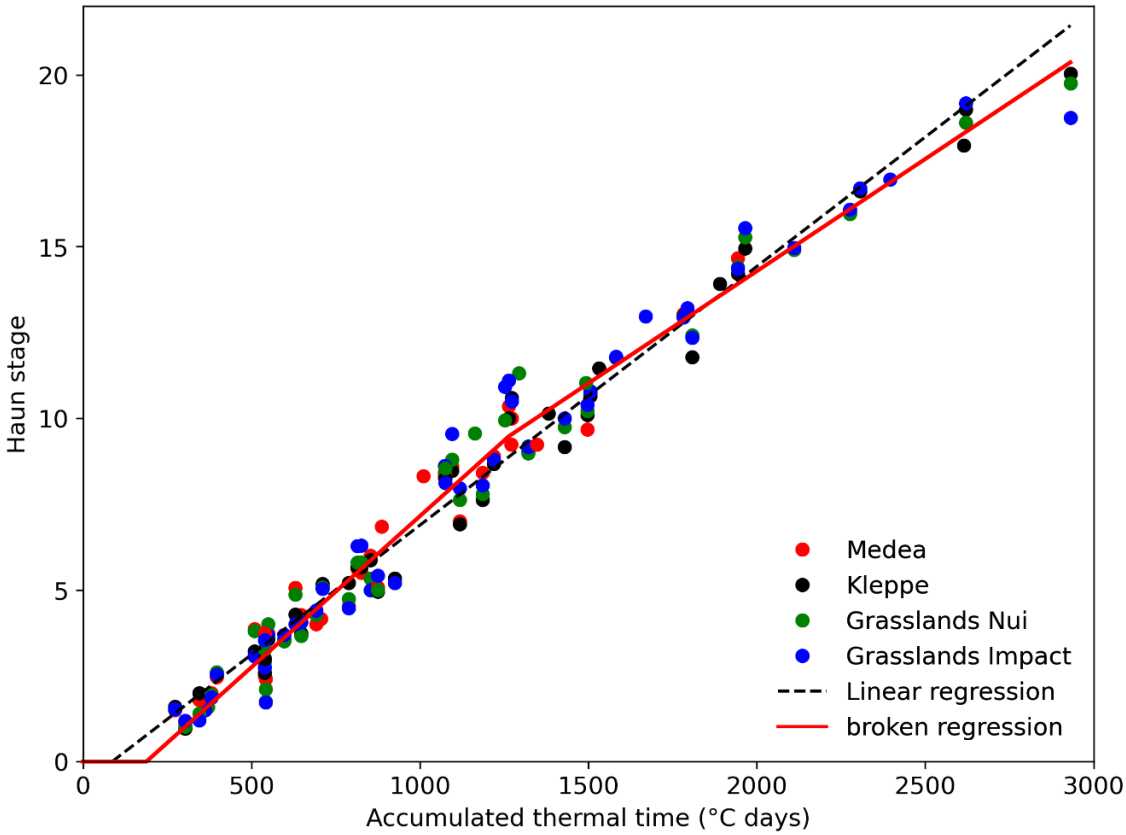


Figure 5.7. Accumulated Haun stage (HS) of four perennial ryegrass genotypes sown on five dates near Lincoln, New Zealand in the 2019/20 growing season. Red line HS = 0 to emergence (188°C days), with a breakpoint at HS 9.5 or 1265°C days where the slope of Line 1 is 0.0088 and the slope of Line 2 is 0.0065, R^2 = 0.96 Black line HS = 0.0075 × x + -0.65, R^2 = 0.97.

5.3.4 Number of main stem leaves

The number of main stem leaves (FLN) produced was analysed using two separate ANOVA because the final two sowings of 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact', into increasing photoperiods, did not achieve 50% of plants flowering. Firstly, 'Medea' was analysed alone to investigate the sowing date influence on FLN on 'Medea' only. Secondly, all genotypes were analysed together for SD1-3 to investigate the genotype by sowing date interaction.

For 'Medea', the number of main stem leaves reduced (P<0.05) from 15.6 (±0.22) in SD1, to 8.5 for SD3, but then increased to ~9.4 for the two later sowing dates ($LSD_{0.05}$ = 0.71) (Figure 5.8, Appendix 10).

For all genotypes, in SD1-3, there was a sowing date (P<0.001, variance ratio = 1231) and genotype (P<0.001, variance ratio = 91) effect but no interaction (P=0.094). For sowing date, the number of main stem leaves reduced from a mean of ~17 (± 0.14) to ~10 as sowing was delayed until mid-winter (Appendix 10). Genotype 'Kleppe' produced the highest number of main stem leaves (mean, 14.6 ± 0.19), 'Medea' produced the fewest (11.6) and 'Grasslands Nui' and 'Grasslands Impact' were intermediate. Full results are presented in Appendix 10.

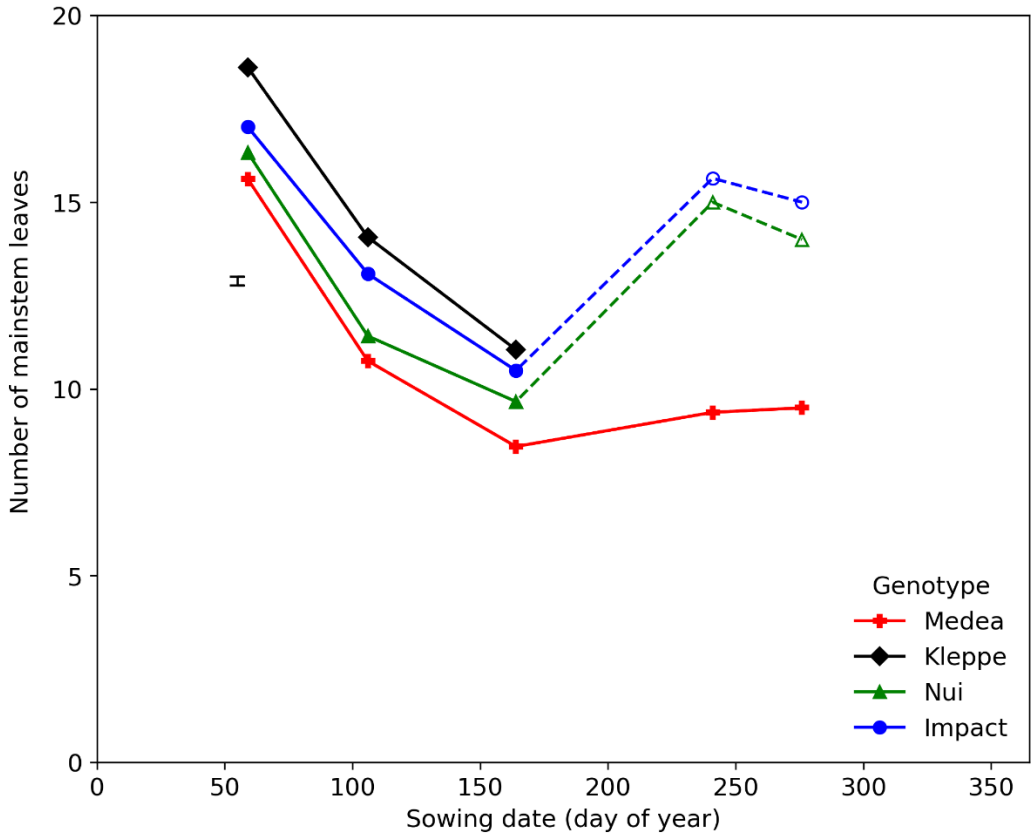


Figure 5.8. Final number of leaves on the main stem of reproductive plants of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand. Solid symbols and lines represent data where more than 50% of plants flowered while open symbols and dashed lines are treatments where less than 50% of plants achieved flowering for reference. Bar = interaction standard error of the mean.

5.3.5 Pattern of organ production – vegetative plants

No 'Medea' treatments remained vegetative as defined by 50% of plants achieving reproductive development. Thus, 'Medea' was not included in the vegetative plant analysis. For 'Kleppe', Grasslands Nui' and 'Grasslands Impact', treatments are included

from SD4 and SD5 where fewer than 50% of plants flowered. For certain treatments, these genotypes had a low number of reproductive plants which were removed from this analysis.

For 'Kleppe', Grasslands Nui' and 'Grasslands Impact', there was a conservative pattern ($P<0.05$) of organ accumulation that could be described using the same two straight line model used in the previous chapter (Figure 5.9). The break occurred at HS 8.1 (±0.26) when 18.7 (±0.42) organs had been produced. The slope of both regression lines was conservative between genotypes at 2.25 (±0.06) and 1.07 (±0.03) for Lines 1 and 2 respectively.

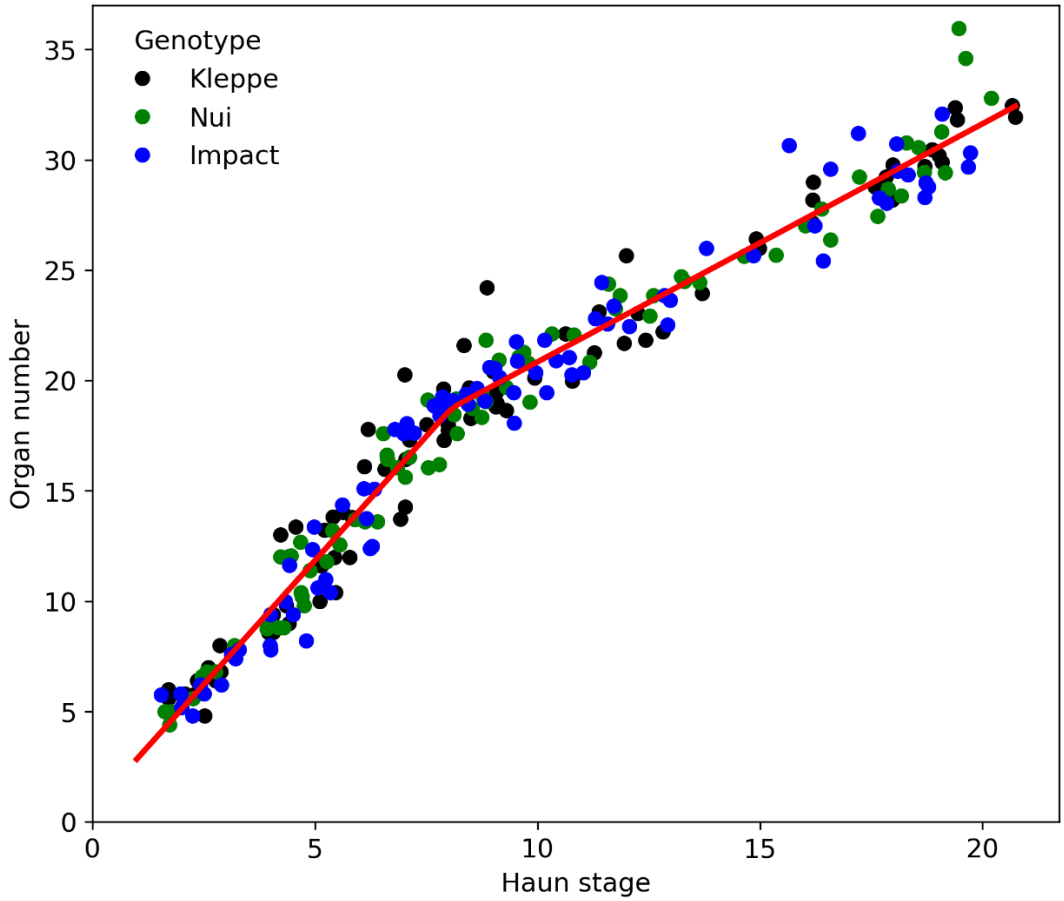


Figure 5.9. Total number of organs (primordium and emerged leaves) against Haun stage on vegetative plants of three perennial ryegrass genotypes grown from five sowing dates near Lincoln, New Zealand in the 2019/20 growing season. Data points are the mean of 3-5 plants dissected weekly. For x less than 8.06 $\mathrm{y} = \mathrm{S1} \times x + \mathrm{y0} - \mathrm{S1} \times x0$, for x greater than 8.06; $\mathrm{y} = \mathrm{S2} \times x + \mathrm{y0} - \mathrm{S2} \times x0$. Where breakpoint x, $(x0)$= 8.08, $(\mathrm{y0})$= 18.7, slope Line 1 $(\mathrm{S1})$= 2.25, slope Line 2 $(\mathrm{S2})$= 1.08, R2 = 0.97.

5.3.6 Pattern of organ production - reproduction

All genotypes followed the expected pattern of organ production when reproductive development occurred (Figure 5.10). Initially organs accumulated at the vegetative rate (2.21/HS), which was conservative (P<0.05) among treatments (Appendix 11), until FI (Table 5.7). Following FI, organs accumulated at the 'reproductive' rate (Appendix 12) until TS, when organ production ceased (Table 5.9). The fitted lines in Figure 5.10 are effectively the mean data from Sections 5.3.7 to 5.3.11 below, while maximum organ numbers are presented in Appendix 13.

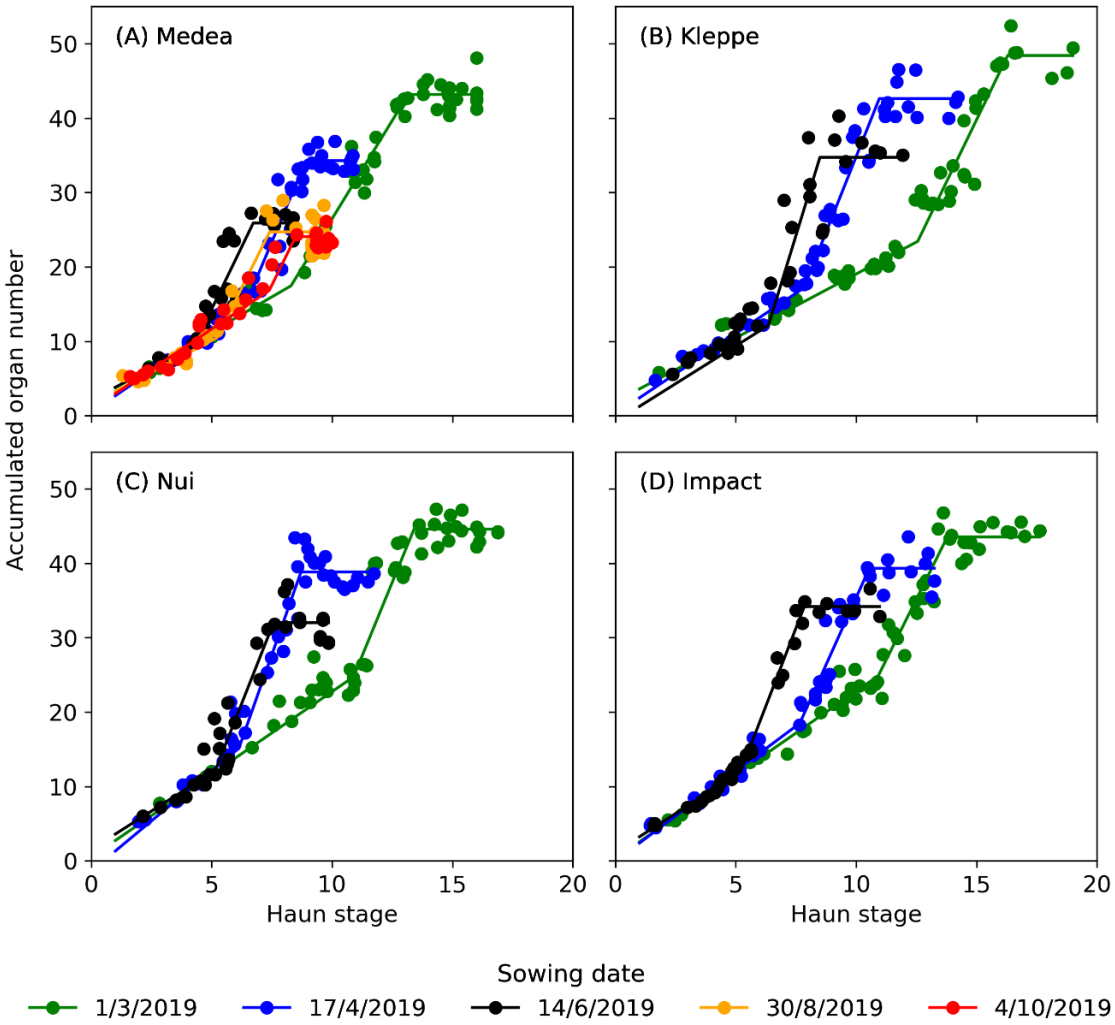


Figure 5.10. Pattern of accumulated organ number of four perennial ryegrass genotypes sown on five dates in 2019 near Lincoln, New Zealand

5.3.7 Floral initiation Haun stage

The floral initiation Haun stage (FI^{HS}) is represented by the breakpoint between the vegetative and reproductive slopes, as per Figure 5.2, with the date of FI presented in Appendix 8.

For 'Medea', sowing date reduced ($P<0.05$) the FI^{HS} from 10.1 to 4.25 as sowing was delayed from SD1 to SD3, subsequently the FI^{HS} increased to 5.95 for SD5 (Table 5.7, Figure 5.10).

For all genotypes within SD1–3, FI^{HS} was influenced by sowing date ($P<0.001$, v.r. = 356) and genotype ($P<0.001$, v.r. = 14) but there was no interaction (Table 5.7). For sowing date, the FI^{HS} reduced from 11.1 at SD1 to 5.24 as sowing was delayed to SD3. The pattern was consistent for all genotypes where 'Medea' and 'Grasslands Nui' achieved FI at the lowest HS while 'Kleppe' was latest and 'Grasslands Impact' intermediate. The minimum FI^{HS} was 4.25. Effectively, earlier sowing dates produced more leaves prior to FI, which delayed development.

Table 5.7. Haun stage at floral initiation of four genotypes of perennial ryegrass sown on five dates near Lincoln, Canterbury, New Zealand in the 2019/20 growing season.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	10.1	12.5	10.7	10.9	11.1
2. 17/04/2019	6.37	7.74	5.9	7.66	6.92
3. 14/06/2019	4.25	6.23	5.15	5.34	5.24
4. 30/08/2019	4.97	-[1]	-	-	4.97
5. 4/10/2019	5.95	-	-	-	5.95
Mean (SD1-3)	6.92	8.84	7.25	7.97	7.76
Mean (SD1-5)	6.33				

Parameter	P value	SEM	$LSD_{0.05}$
Medea only[2]			
Sowing date	<0.001	0.501	1.635
All genotypes – SD1-3 only[3]			
Sowing date	<0.001	0.166	0.652
Genotypes	<0.001	0.231	0.686
Sowing date * genotype	0.317	0.392	1.127
Means within a sowing date		0.402	1.189

[1] –genotype by sowing date combination that did not achieve 50% of plants flowering

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

5.3.8 Photoperiod at FI^{HS}

The Pp at FI^{HS} was identified by plotting FI^{HS} against the Pp at FI^{HS} where stacking of FI^{HS} at the same Pp suggests a threshold Pp for FI (Pp_{base}). For 'Medea', 'Grasslands Nui' and 'Kleppe', slight increases in the Pp at FI^{HS} occurred as sowing was delayed from SD1 to SD2 but effectively demonstrated a Pp_{base} response was required for FI (Figure 5.11). For 'Medea' the Pp at FI for SD1 was 10.7 h, similar to that of 'Grasslands Nui' while for 'Kleppe' a minimum Pp of 12.3 h was required for FI. 'Grasslands Impact' showed a shift in Pp from 10.5 h in SD1 to 12.4 h in SD2. Sowing dates 3-5 delayed FI and therefore increased the Pp at FI^{HS} as plants reached the end of their juvenile phase (HS5) and were able to respond to Pp.

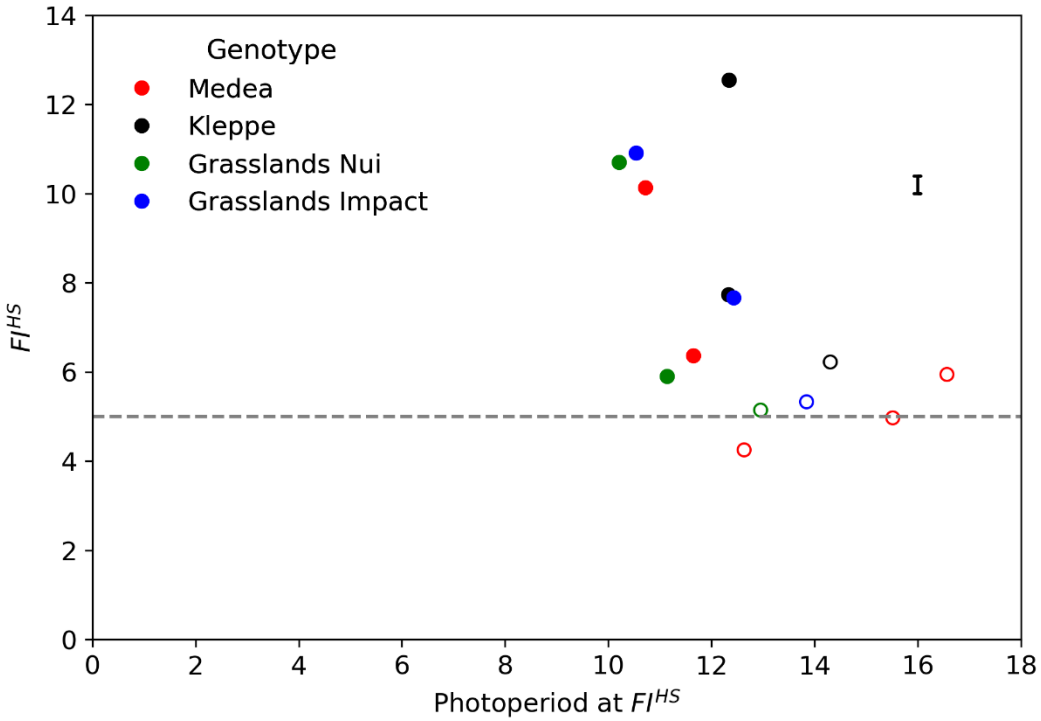


Figure 5.11. Floral initiation Haun stage (FI^{HS}) against the photoperiod at FI^{HS} of four perennial ryegrass genotypes sown on five dates at Lincoln, New Zealand. Closed symbols are sowing dates (SD) 1 (1/03/2019) and 2 (17/04/2019) while open symbols are SD3,4 and 5 which are delayed by a juvenile phase of 5 Haun stages represented by the constant grey line. Bar is the SEM for genotype x sowing date interaction for FI^{HS}.

5.3.8.1 Modelling FI

Assuming V_{base} has been achieved (see Chapter 4) and HS5 has been reached, FI occurs when Pp_{base} is reached in a lengthening Pp.

5.3.9 Duration from FI-TS

The duration from FI^{HS} to TS^{HS} was reduced (P<0.05) by SD from 3.1 HS for SD1 to 2.5 HS at SD3, for the mean of all genotypes (Table 5.7). There was no effect of genotype (P=0.297) and no interaction (P=0.942).

For 'Medea' only, there was no sowing date effect (P=0.850) when analysed across five sowing dates.

Table 5.8. Duration, expressed in Haun stages, from floral initiation to terminal spikelet formation for four genotypes of perennial ryegrass sown on five dates near Lincoln, Canterbury, New Zealand.

	Genotype				Sowing
Sowing date	Medea	Kleppe	Grasslands Nui	Grasslands Impact	date mean
1. 1/03/2019	2.97	3.71	2.76	3.04	3.12
2. 17/04/2019	2.54	3.44	3.01	2.89	2.97
3. 14/06/2019	2.48	2.65	2.42	2.48	2.51
4. 30/08/2019	2.24	-[1]	-	-	2.24
5. 4/10/2019	2.20	-	-	-	2.20
Mean (SD1-3)	2.66	3.27	2.73	2.80	2.87
Mean (SD1-5)	2.49				

Parameter	P value	SEM	$LSD_{0.05}$
Medea only[2]			
Sowing date	0.850	0.537	1.751
All genotypes – SD1–3 only[3]			
Sowing date	0.004	0.057	0.223
Genotype	0.297	0.237	0.705
Sowing date * genotype	0.942	0.361	1.068
Means within a sowing date		0.411	1.222

[1] –genotype by sowing date combination that did not achieve 50% of plants flowering
[2] Medea analysed alone across five sowing dates
[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

The duration from FI^{HS} to TS^{HS}, expressed in Haun stages, was well described by regression analysis were $TS^{HS} = FI^{HS} \times 1.12 (\pm 0.03) + 1.86 (\pm 0.25)$ (R^2=0.98, P<0.001) (Figure 5.12). This suggests a constant relationship that increased from ~HS1.8 by 0.12 as the FI^{HS} increased.

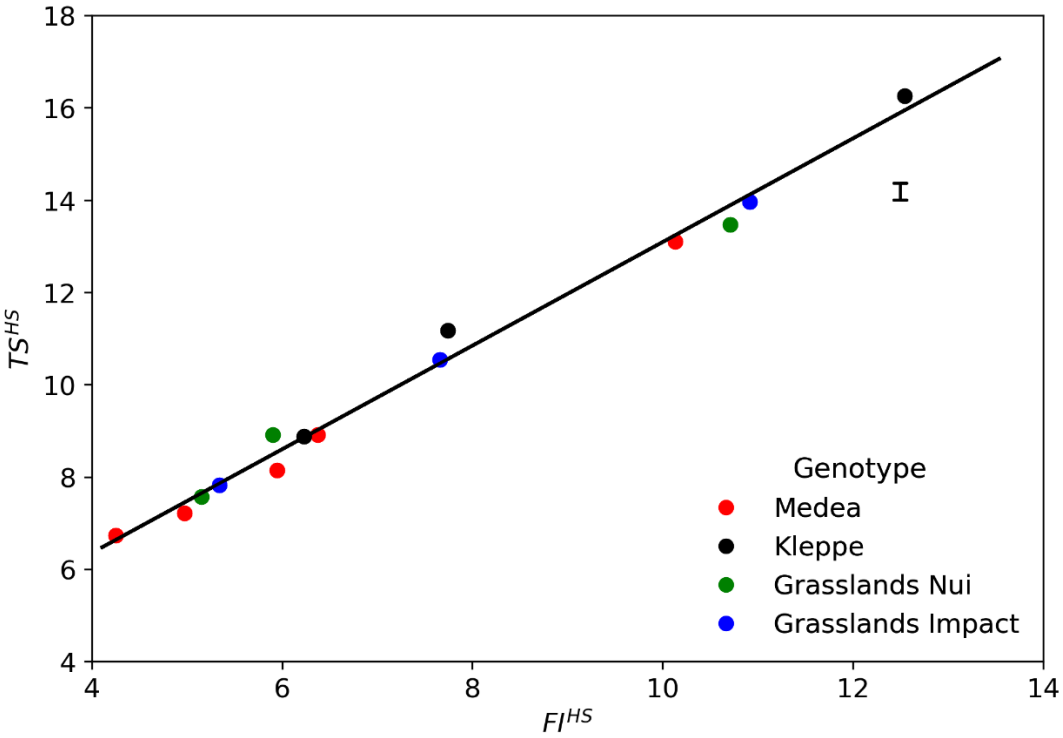


Figure 5.12. Time, represented as Haun stage, from floral initiation Haun stage (FI^{HS}) until terminal spikelet Haun stage (TS^{HS}) of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand. Each data point is the mean of three replicates. Sowing dates included if more than 50% of plants were reproductive. Regression (black line), $TS^{HS} = FI^{HS} \times 1.12$ (±0.03) + 1.86 (±0.25) (R^2=0.98, P<0.001), bar is SEM for the genotype by genotype interaction, 0.361.

In the field, higher FI^{HS} resulted from earlier sowing when plants had a longer time to produce leaves, thus leading to a higher FI^{HS}. It is unlikely that delayed sowing date alone provides a direct mechanism for reducing the duration from FI^{HS}-TS^{HS}. It is more likely that increasing Pp reduces the duration from FI^{HS}-TS^{HS} for later sowing dates where FI occurred at a later calendar date. To account for this, the duration from FI^{HS}-TS^{HS} was plotted against the mean Pp between FI and TS for each genotype by sowing date treatment (Figure 5.13). 'Medea', 'Grasslands Nui' and 'Grasslands Impact' produced negative relationships where FI-TS duration decreased as Pp increased. For 'Kleppe' the slope response was greater at -0.75 (±0.21)/hr of Pp increase.

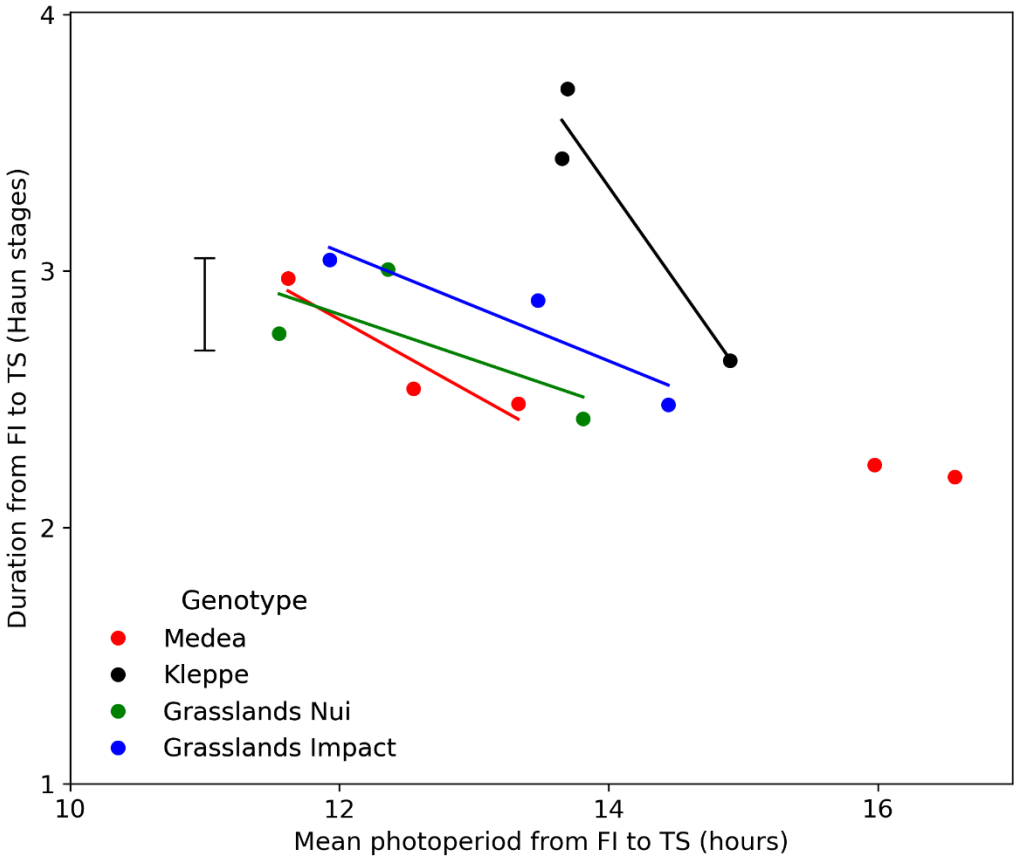


Figure 5.13. Duration in Haun stages, from floral initiation (FI) to terminal spikelet (TS) presented over mean photoperiod (Pp) between FI and TS of four perennial ryegrass genotypes sown on five dates near Lincoln, New Zealand in the 2019/20 growing season. 'Medea', = -0.29 (±0.11) × Pp + 6.32 (± 1.38) (R^2=87.5), 'Kleppe' = -0.75 (±0.21) × Pp + 13.8 (± 3.00) (R^2=0.93), 'Grasslands Nui' = -0.18 (±0.18) × Pp + 4.96 (± 2.32) (R^2=0.48) and 'Grasslands Impact' = -0.21 (±0.0.9) × Pp + 5.63 (± 1.14) (R^2=0.86). Bar is SEM for the genotype by sowing date interaction, 0.361.

5.3.10 Time of terminal spikelet Haun stage

The TS^{HS} was influenced by sowing date (P<0.001, v.r. = 769), genotype (P<0.001, v.r. = 95) and their interaction (P=0.002, v.r. = 5.4). For SD1-3, the TS^{HS} reduced as sowing date was delayed for all genotypes where, TS^{HS} was lower for 'Medea' and 'Grasslands Nui' and largest in 'Kleppe' (Table 5.9), a pattern consistent with the FI^{HS} (Table 5.7).

For 'Medea' the TS^{HS} reduced (P<0.05) from 13.1 (± 0.29) to 6.73 as sowing was delayed from SD1-3 and increased to 8.14 as sowing was further delayed into the spring (Table 5.9).

Table 5.9. Haun stage at which terminal spikelet occurred on four genotypes of perennial ryegrass sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	13.1	16.3	13.5	14.0	14.2
2. 17/04/2019	8.91	11.2	8.91	10.5	9.88
3. 14/06/2019	6.73	8.88	7.57	7.82	7.75
4. 30/08/2019	7.22	-[1]	-	-	7.22
5. 4/10/2019	8.14	-	-	-	8.14
Mean (SD1-3)	9.58	12.1	9.98	10.8	10.6
Mean (SD1-5)	8.82				
Parameter			P value	SEM	$LSD_{0.05}$
Medea only[2]					
Sowing date			<0.001	0.291	0.949
All genotypes – SD1-3 only[3]					
Sowing date			<0.001	0.167	0.465
Genotype			<0.001	0.161	0.338
Sowing date * genotype			0.002	0.294	0.614
Means within a sowing date				0.279	0.586

[1] – genotype did not achieve 50% of plants flowering from this sowing date.
[2] Medea analysed alone across five sowing dates
[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

5.3.10.1 Predicting TS from FI^{HS} and Pp

The HS duration from FI^{HS}-TS^{HS} can be predicted by multiplying the Pp at FI^{HS} by the Pp response.

5.3.11 Leaves post TS

For all genotypes, in SD1-3, genotype influenced (P<0.05) the number of leaves produced post TS, but there was no sowing date or interaction effect. Specifically, 'Medea' produced fewer (2.03 ± 0.14) leaves post TS than 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact' which were conservative at 2.57 (Table 5.10).

For 'Medea', there was no difference (P=0.079) across five sowing dates with a mean of 1.9 leaves remaining to appear after terminal spikelet formation (Table 5.10).

Table 5.10. Number of leaves remaining to emerge post terminal spikelet production of four perennial ryegrass genotypes sown on five dates near Lincoln, New Zealnd in the 2019/2020 growing season.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	2.52	2.32	2.87	3.07	2.70
2. 17/04/2019	1.84	2.88	2.51	2.54	2.44
3. 14/06/2019	1.72	2.17	2.09	2.67	2.17
4. 30/08/2019	2.15	-[1]	-	-	2.15
5. 4/10/2019	1.35	-	-	-	1.35
Mean (SD1-3)	2.03	2.47	2.49	2.76	2.44
Mean (SD1-5)	1.92				
Parameter			P value	SEM	$LSD_{0.05}$
Medea only[2]					
Sowing date			0.079	0.25	0.817
All genotypes – SD1-3 only[3]					
Sowing date			0.256	0.192	0.755
Genotype			0.012	0.136	0.405
Sowing date * genotype			0.213	0.281	0.851
Means within a sowing date				0.236	0.702

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

FLN was analysed by linear regression for the two genotype groups identified via ANOVA, using TS^{HS} as the descriptor. For 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact' the relationship was described with a y-axis intercept of 2.06 (±0.42) and a slope of 1.04 (±0.04) (Figure 5.14). This suggests that regardless of the TS^{HS} there were ~2 leaves remaining to emerge at TS formation (FLN = TS^{HS} *1.04 (±0.04) + 2.06 (±0.42) (R^2 = 0.99). For, 'Medea', the relationship was described with a y-axis intercept of 0.89 (±0.69) and a slope of 1.12 (±0.08). Thus, both analysis methods indicate 'Medea' produced fewer leaves post TS than the other genotypes.

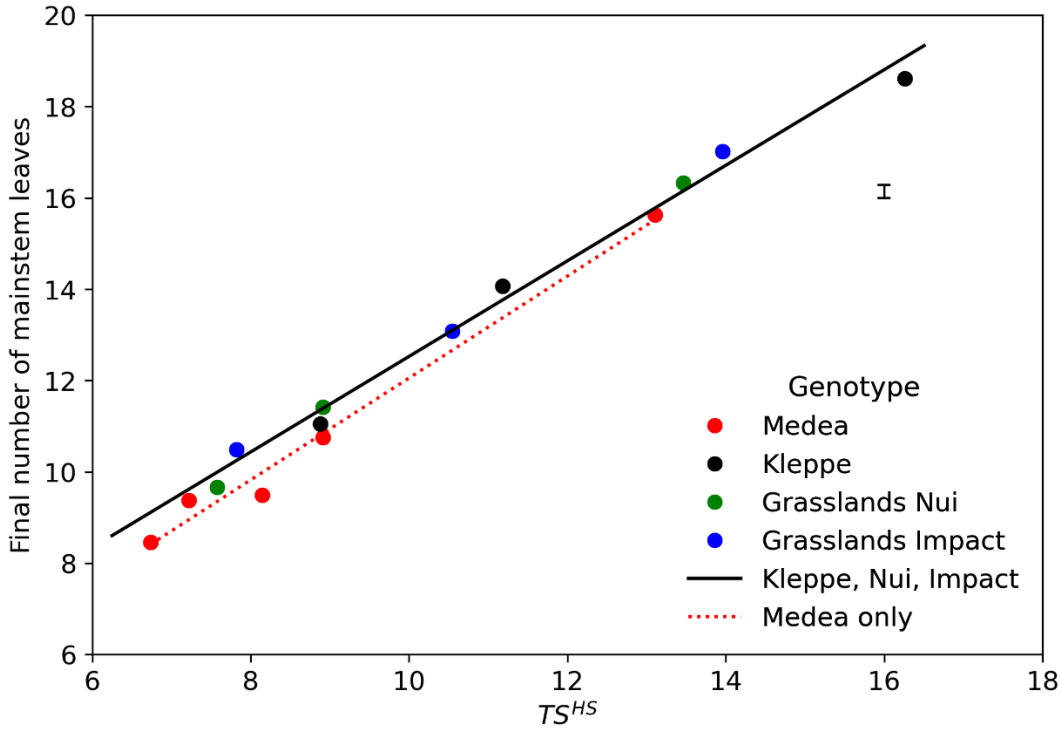


Figure 5.14. Number of main stem leaves in relation to the Haun stage at which terminal spikelet formation occurred on four genotypes of perennial ryegrass sown on five dates near Lincoln, Canterbury, New Zealand. Black line is FLN = TS^{HS} × 1.04 (± 0.04) + 2.06 (± 0.42) (R^2 = 0.99). Red line is FLN = TS^{HS} × 1.12 + 0.89 (±0.69) (R^2 = 0.99).

5.3.11.1 Modelling leaf number post TS

The number of leaves to emerge following the formation of TS is 2 or 2.5.

5.3.12 Leaves produced post FI

The number of leaves produced post FI (LN_{postFI}) effectively combines the FI-TS and TS-FL phases into a single calculation. For 'Medea' there was no difference (P=0.298) in the LN_{postFI} between sowing dates.

When all genotypes were analysed together, genotype (P<0.05) influenced LN_{postFI} with a trend (P=0.067) that indicated delayed sowing reduced LN_{postFI}. There was no interaction (P=0.629, Table 5.11). For genotype, 'Medea' (4.69 ±0.25) produced fewer leaves post FI than 'Grasslands Impact' (5.56) and 'Kleppe' (5.74) while 'Grasslands Nui' (5.22), 'Grasslands Impact' and 'Kleppe' were not different to each other. There was a trend (P=0.067) for sowing date to reduce the LN_{postFI} from 5.82 (±0.24) at SD1 to 4.67 at SD3 (Table 5.11).

Table 5.11. Leaves produced post floral initation of four perennial ryegrass genotypes sown on five dates near Lincoln, New Zealand in the 2019/20 growing season.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	5.49	6.07	5.63	6.10	5.82
2. 17/04/2019	4.38	6.33	5.51	5.42	5.41
3. 14/06/2019	4.21	4.82	4.51	5.15	4.67
4. 30/08/2019	4.40	-[1]	-	-	4.40
5. 4/10/2019	3.55	-	-	-	3.55
Mean (SD1-3)	4.69	5.74	5.22	5.56	2.47
Mean (SD1-5)	4.41				
Parameter			P value	SEM	$LSD_{0.05}$
Medea only[2]					
Sowing date			0.298	0.578	1.886
All genotypes – SD1-3 only[3]					
Sowing date			0.067	0.243	0.954
Genotype			0.036	0.245	0.728
Sowing date * genotype			0.629	0.440	1.299
Means within a sowing date				0.423	1.26

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

5.3.13 Final leaf number and photoperiod

Linear regression was used to investigate whether Pp influenced FLN at sowing, emergence, FL, DR, TS or final leaf ligule for all genotypes (Figure 5.15). Saturation of the Pp response was calculated by using a minimum FLN of 9 and substituting to find x in the linear regression equations. The minimum FLN of 9 was selected as the mean of genotype 'Medea' from SD3, 4 and 5 and was not different to the minimum achieved in Chapters 3 and 4 in controlled environment chamber experiments.

The number of main stem leaves decreased ($P<0.05$) as the Pp at time of sowing and emergence decreased for all genotypes (Figure 5.15A,B). The implication is that all genotypes produced more leaves as the mechanism to delay development until appropriate (increasing Pp) conditions for FI occurred.

For 'Medea', FLN reduced as Pp at sowing and emergence shortened (Figure 5.15A,B) and treatments reached FI in an increasing Pp. The Pp relationship at sowing or emergence, allows for the estimation of FLN based on coefficients derived from linear regression. However, practically, such estimates would require adjustments based on accumulated temperature and phyllochron up-until the directional change in Pp. As Pp increased for the mid winter to spring sowings, FLN was constant at ~9. Thus, SD4 and SD5 were excluded from regression analyses, which aimed to describe the mechanisms responsible for reductions in FLN. Coefficients of determination (R^2 values) were above 0.95 for all developmental timings which suggests Pp was pivotal in controlling FLN. The Pp at TS provided the highest R^2 value and was the only analysis where $P<0.05$ (Table 5.12.). This suggests Pp at TS was the most accurate predictor of the commitment of FLN. The Pp at FI for SD1 was 10.7 h (Figure 5.15C) and sets the base Pp requirement for 'Medea' while the calculated Pp_{sat} was 13.9 h.

For 'Kleppe', FLN reduced as Pp at sowing and emergence decreased, consistent with 'Medea' (Figure 5.15A,B). SD1 and 2 reached FI at a Pp of 12.3 h while SD3 required 14.3 h, which suggests a minimum Pp of 12.3 h was required for FI (Figure 5.15C). The visual fit for linear regression appeared to improve as the timing increased from FI until TS. However, this was not supported by the R^2 values, largely due to only having three data

points. At TS, the slope was -14 leaves/h of Pp suggesting a Pp_{sat} of 15.5 h where TS formation will occur as quickly as possible (Figure 5.15E, Table 5.12).

The 'Grasslands Nui' Pp response was aligned (parallel) to 'Kleppe' where 10.3 h was required for FI for SD1 and 11.1 h for SD2, compared with the 10.7 required by 'Medea' (Figure 5.15C). However, following FI, 'Grasslands Nui' took longer to reach TS than 'Medea'. Thus, it produced more leaves and had a delayed final leaf appearance (Section 5.3.14). This response was quantified by the lower regression slope at all timings between FI and TS. For example, at TS the reduction in the number of leaves/h Pp (regression slope) for 'Medea' was faster at -4.81 leaves/h Pp compared with -3.56 for 'Grasslands Nui' (Table 5.12) (Figure 5.15C,D,E). For 'Grasslands Nui' a saturating Pp of 14.7 h was identified by using the Pp at TS (Table 5.12).

'Grasslands Impact' demonstrated a comparable FI response to 'Medea' followed by a strong linear reduction as Pp increased at all developmental stages from FI until final leaf emergence (Figure 5.15C,D,E). The Pp required for FI in SD1 was 10.5 h, which aligned with 'Medea' and 'Grasslands Nui' (Figure 5.15C). However, the reduction in leaves/h Pp was slower for 'Grasslands Impact' (-1.98) during the ear development phase (Medea = -3.73 and Grasslands Nui = 2.24). This allowed the production of further leaves at shorter Pps and subsequently delayed final leaf appearance. For 'Grasslands Impact' a saturating Pp of 15.5 h was identified by using the Pp at TS (Table 5.12).

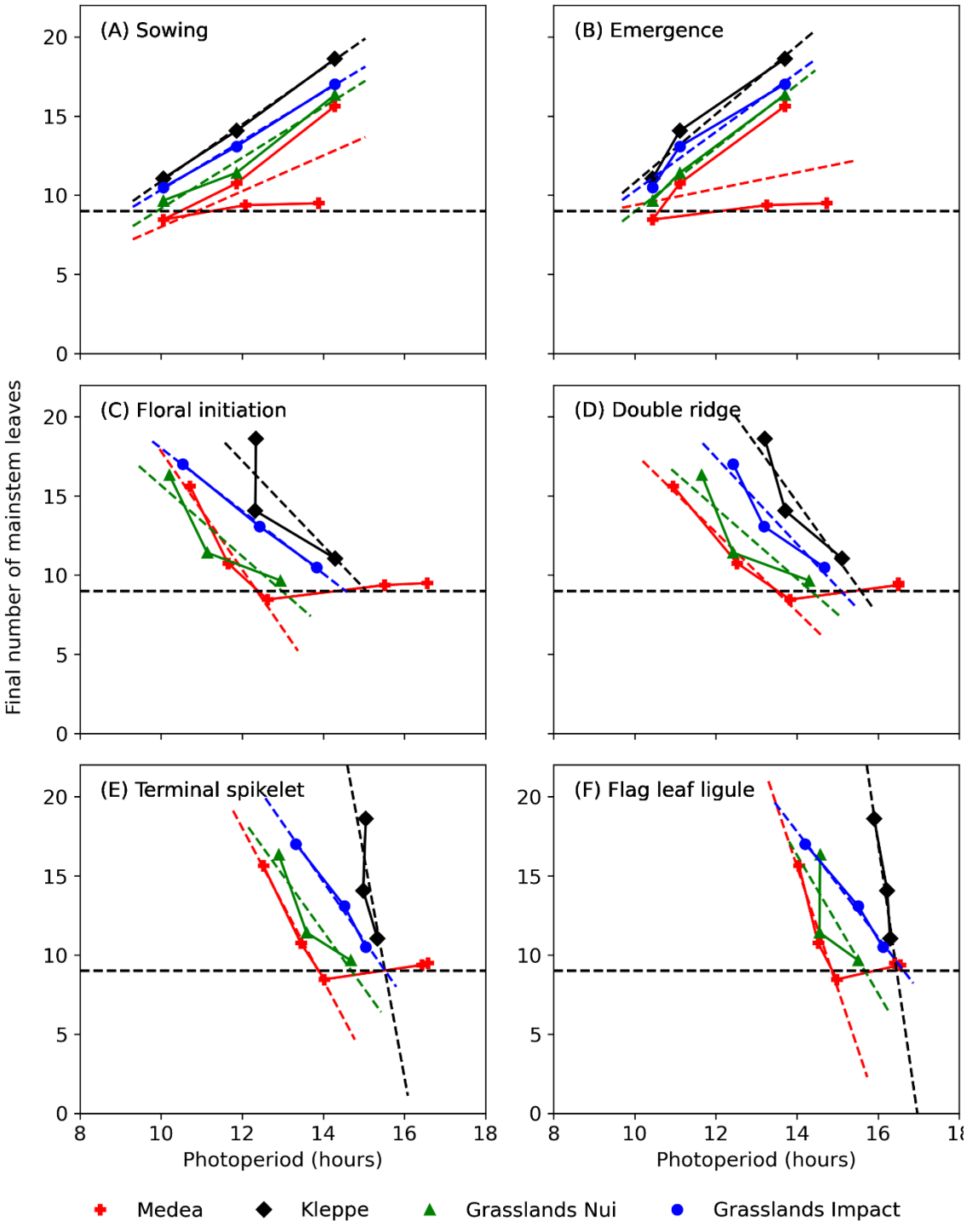


Figure 5.15. Number of main stem leaves produced in relation to the photoperiod (Pp) at eight different developmental timings of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand. Dashed lines represent linear regression for data presented, except for 'Medea' in C,D,E and F where regression represents the first three sowing dates only. Grey dashed line is constant 4 leaves which represents the minimum possible at Pp saturation. Data only presented if more than 50% of plants flowered for a specific sowing date.

Table 5.12. Parameters from linear regression analysis used to describe the final leaf number in relation to photoperiod (Pp) at six developmental times of four ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand in the 2019/20 growing season. Saturating Pp calculated as the point where the regression line reaches nine leaves (the minimum possible leaf number).

Genotype	Timing	Intercept	Slope (leaves/h Pp)	R^2	P value	Saturating Pp (hours)
Medea	Sowing	-3.26	1.13	0.27	0.21	10.9
	Emergence	4.20	0.52	0.19	0.59	9.28
	FI	55.2	-3.73	0.95	0.14	12.4
	DR	42.7	-2.50	0.98	0.10	13.5
	TS	75.8	-4.81	0.99	0.05	13.9
	Final leaf	123	-7.67	0.96	0.13	14.9
Kleppe	Sowing	-7.03	1.79	1.00	0.02	8.95
	Emergence	-10.8	2.16	0.91	0.13	9.17
	FI	49.4	-2.68	0.64	0.41	15.1
	DR	64.6	-3.57	0.86	0.24	15.6
	TS	226	-13.98	0.47	0.52	15.5
	Final leaf	299	-17.61	0.95	0.15	16.5
Nui	Sowing	-6.84	1.60	0.93	0.12	9.90
	Emergence	-11.1	2.00	0.99	0.04	10.0
	FI	38.0	-2.24	0.81	0.29	13.0
	DR	41.0	-2.23	0.77	0.32	14.3
	TS	61.4	-3.56	0.85	0.25	14.7
	Final leaf	77.4	-4.37	0.48	0.51	15.7
Impact	Sowing	-5.11	1.55	1.00	0.02	9.13
	Emergence	-8.38	1.86	0.91	0.13	9.32
	FI	37.8	-1.98	1.00	0.02	14.6
	DR	50.3	-2.74	0.91	0.19	15.1
	TS	66.3	-3.69	0.99	0.07	15.5
	Final leaf	64.6	-3.34	0.99	0.06	16.6

5.3.14 Date of final leaf emergence

For analysis, dates were converted into 'Julian days', subjected to ANOVA and back converted into dates, thus standard errors and the LSD are presented in days.

Final leaf emergence date was affected (P<0.001) by a sowing date by genotype interaction (Table 5.13). Genotype, influenced the time of final leaf emergence while delayed sowing usually delayed the emergence of the final leaf. The exceptions were 'Kleppe' and 'Grasslands Nui' where the final leaf from SD 1 and 2 emerged at similar times within the genotype (Table 5.13).

Table 5.13. Date of emergence for the final leaf of four genotypes of perennial ryegrass sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	7/10/2019	24/11/2019	17/10/2019	26/10/2019	26/10/2019
2. 17/04/2019	16/10/2019	23/11/2019	22/10/2019	5/11/2019	1/11/2019
3. 14/06/2019	25/10/2019	29/11/2019	5/11/2019	20/11/2019	12/11/2019
4. 30/08/2019	11/12/2019	-[1]	-	-	11/12/2019
5. 4/10/2019	31/12/2019	-	-	-	31/12/2019
Mean (SD1-3)	16/10/2019	25/11/2019	25/10/2019	6/11/2019	2/11/2019
Mean (SD1-5)	11/11/2019				
Parameter			P value	SEM	$LSD_{0.05}$
Medea only[2]					
Sowing date			<0.001	1.535	5.005
All genotypes – SD1-3 only[3]					
Sowing date			<0.001	0.857	3.366
Genotype			<0.001	1.010	3.002
Sowing date * genotype			<0.001	1.741	5.114
Means within a sowing date				1.750	5.199

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

5.3.15 Ear emergence dates

For SD1–3 only, full ear emergence was influenced (P<0.001) by both sowing date and genotype but not their interaction. In most cases, genotype set the broad timeframe of ear emergence where 'Medea' was first, followed by 'Grasslands Nui', 'Grasslands Impact' and 'Kleppe'. For mean ear emergence SD1 and SD2 were not different, but SD3 was delayed (Table 5.14).

For 'Medea', SD1 and SD2 achieved ear emergence at the same time, while all other sowing dates were different from each other (Table 5.14).

Table 5.14. Full ear emergence date of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	6/11/2019	7/12/2019	14/11/2019	26/11/2019	21/11/2019
2. 17/04/2019	9/11/2019	7/12/2019	11/11/2019	30/11/2019	21/11/2019
3. 14/06/2019	17/11/2019	17/12/2019	26/11/2019	6/12/2019	1/12/2019
4. 30/08/2019	27/12/2019	-[1]	-	-	27/12/2019
5. 4/10/2019	12/01/2020	-	-	-	12/01/2020
Mean (SD1-3)	10/11/2019	10/12/2019	17/11/2019	30/11/2019	24/11/2019
Mean (SD1-5)	2/12/2019				

Parameter	P value	SEM	$LSD_{0.05}$
Medea only[2]			
Sowing date	<0.001	1.265	4.125
All genotypes – SD1–3 only[3]			
Sowing date	<0.001	0.385	1.511
Genotype	<0.001	0.927	2.755
Sowing date * genotype	0.157	1.443	4.254
Means within a sowing date		1.606	4.773

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

5.3.16 Flowering date

For SD1–3 only, flowering date was influenced (P<0.001) by both sowing date and genotype but not their interaction. 'Medea' reached flowering earliest followed by 'Grasslands Nui', 'Grasslands Impact' and 'Kleppe'. For sowing date means, flowering followed the sowing date pattern. Full data are shown for interpretation in Table 5.15.

For 'Medea', flowering followed the sowing date pattern meaning this genotype responded primarily to Pp (Table 5.15).

Table 5.15. Flowering date of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	23/11/2019	17/12/2019	27/11/2019	6/12/2019	3/12/2019
2. 17/04/2019	30/11/2019	18/12/2019	1/12/2019	9/12/2019	7/12/2019
3. 14/06/2019	2/12/2019	27/12/2019	7/12/2019	15/12/2019	12/12/2019
4. 30/08/2019	14/01/2020	-[1]	-	-	14/01/2020
5. 4/10/2019	25/01/2020	-	-	-	25/01/2020
Mean (SD1-3)	28/11/2019	20/12/2019	2/12/2019	10/12/2019	25/11/2019
Mean (SD1-5)	19/12/2019				

Parameter	P value	SEM	$LSD_{0.05}$
Medea only[2]			
Sowing date	<0.001	1.434	4.676
All genotypes – SD1–3 only[3]			
Sowing date	0.003	0.805	3.161
Genotype	<0.001	0.956	2.841
Sowing date * genotype	0.595	1.645	4.831
Means within a sowing date		1.656	4.922

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

5.3.17 Thermal time duration from final leaf emergence to flowering

For SD1–3, overall duration in °C days from final leaf emergence flowering (anthesis) (FL-AN) was affected ($P<0.05$) by the interaction of sowing date and genotype. For 'Medea', 'Kleppe' and 'Grasslands Nui' the duration was constant within genotype and among sowing dates. 'Medea' (600 ± 29°C days) was not different to 'Grasslands Nui' at 557°C days, while 'Kleppe' was faster at 416°C days. For 'Grasslands Impact' the duration from final leaf emergence until flowering decreased from 619 to 416°C days as sowing was delayed from SD1 to SD3 (Table 5.16).

For 'Medea', analysed for all sowing dates, the duration in thermal time from final leaf emergence until flowering reduced ($P<0.05$) as sowing was delayed. SD1, 2 and 3 were not different (mean = 600 ±29.5) but SD1 and SD2 took longer than SD4, and SD5 was faster than all other sowing dates (Table 5.16).

Overall, the longest duration for 'Medea', and 'Grasslands Impact' was ~600°C days for early sowing dates, which reduced to ~400°C days as sowing was delayed.

Table 5.16. Thermal time (°C days), based on screen air temperature above a base temperature of 0°C, from final leaf appearance until flowering of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	613	383	574	619	547
2. 17/04/2019	624	423	579	548	543
3. 14/06/2019	564	442	520	416	486
4. 30/08/2019	511	-[1]	-	-	511
5. 4/10/2019	392	-	-	-	392
Mean (SD1-3)	600	416	557	527	525
Mean (SD1-5)	541				
Parameter			P value	SEM	$LSD_{0.05}$
Medea only[2]					
Sowing date			0.004	30.9	100.9
All genotypes – SD1–3 only[3]					
Sowing date			0.04	12.3	48.1
Genotype			<.001	17.9	53.1
Sowing date * genotype			0.03	29.5	86.4
Means within a sowing date				30.9	91.9

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

To investigate if the reduction in Tt from FL-AN for 'Medea' and 'Grasslands Impact' was a growth or developmental response, potential dry matter (DM) production and the influence of Pp on the FL-AN duration were considered.

Firstly, potential DM (PDM) was calculated in daily timesteps for the duration from final leaf emergence (GS 39) until flowering (GS 65), for the duration from FL-AN. Daily potential growth rates were calculated using Equation 28. The radiation use efficiency (RUE) was set at 1.1 g DM/MJ and f(T) had a parabolic increase from zero at 0°C to 1 at the optimum of 20°C and back to zero at 40°C (Jamieson *et al.* 1998b).

$$PDM = R \times RUE \times f(T)$$ Equation 28

Where: R is solar radiation in MJ/m^2
RUE is the crop's radiation use efficiency (1.1 g DM/MJ)
f(T) is a factor (0–1) calculated by 1-0.0025*(T_{opt}-T_{mean})^2.
Where: T_{opt} was 20°C
T_{mean} is the mean daily temperature

Potential mean daily growth rate was affected (P<0.001) by the genotype by sowing date interaction where 'Kleppe' had a constant growth rate of 211 (±2.42) kg DM/ha/d across the three sowing dates (Table 5.17). The mean daily growth rate for 'Medea' increased in all of the first four sowing dates from 170 to 214 kg DM/ha/d while SD4 (214 kg DM/ha/d) and SD5 (221 kg DM/ha/d) were the same ($LSD_{0.05}$ = 7.24). 'Grasslands Nui' showed a constant increase in DM production where SD1 (182 kg DM/ha/d) and SD3 (191 kg DM/ha/d) were different from each other while SD2 was not different to either at 187 kg DM/ha/d. 'Grasslands Impact' showed constant potential growth rates between SD1 and SD2 which increased at SD3.

When comparing growth rates and FL-AN duration, many of the same trends are present. For example, in 'Grasslands Impact' the differences between growth rate and FL-AN are not different where SD1 and SD2 are the same but SD3 is different. In 'Kleppe' there was no difference between sowing dates for either PDM production or FL-AN. However, for 'Medea' and 'Grasslands Nui' there are contrasts between the data sets. For 'Grasslands Nui' there was no difference in FL-AN, but differences in potential growth rates. For 'Medea', there were reductions in FL-AN where SD1, SD2 and SD3 were similar but SD1 and SD2 were longer than SD4, while SD5 was shorter than all sowing dates. For 'Medea' the potential DM for SD1–3 were different from each other while SD4 and SD5 were not different, thus producing a different pattern and therefore unlikely to be the mechanism for changes in FL-AN.

Table 5.17. Calculated potential mean daily growth rates in kg DM/ha of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	170	212	182	192	189
2. 17/04/2019	182	211	187	194	194
3. 14/06/2019	190	211	191	211	201
4. 30/08/2019	214	-[1]	-	-	214
5. 4/10/2019	221	-	-	-	221
Mean (SD1-3)	181	211	187	199	
Mean (SD1-5)	196				
Parameter			P value	SEM	$LSD_{0.05}$
Medea only[2] Sowing date			<0.001	2.22	7.24
All genotypes – SD1–3 only[3]					
Sowing date			0.019	1.62	6.34
Genotype			<.001	1.20	3.57
Sowing date * genotype			<.001	2.42	7.30
Means within a sowing date				2.08	6.18

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

When PDM was plotted against FL-AN, for two durations, 1) final leaf to full head emergence (Figure 5.16A,B) and 2) FL-AN (Figure 5.16C,D) the general trend supported a relationship whereby shorter durations from FL-AN occurred at greater rates of PDM (Figure 5.16).

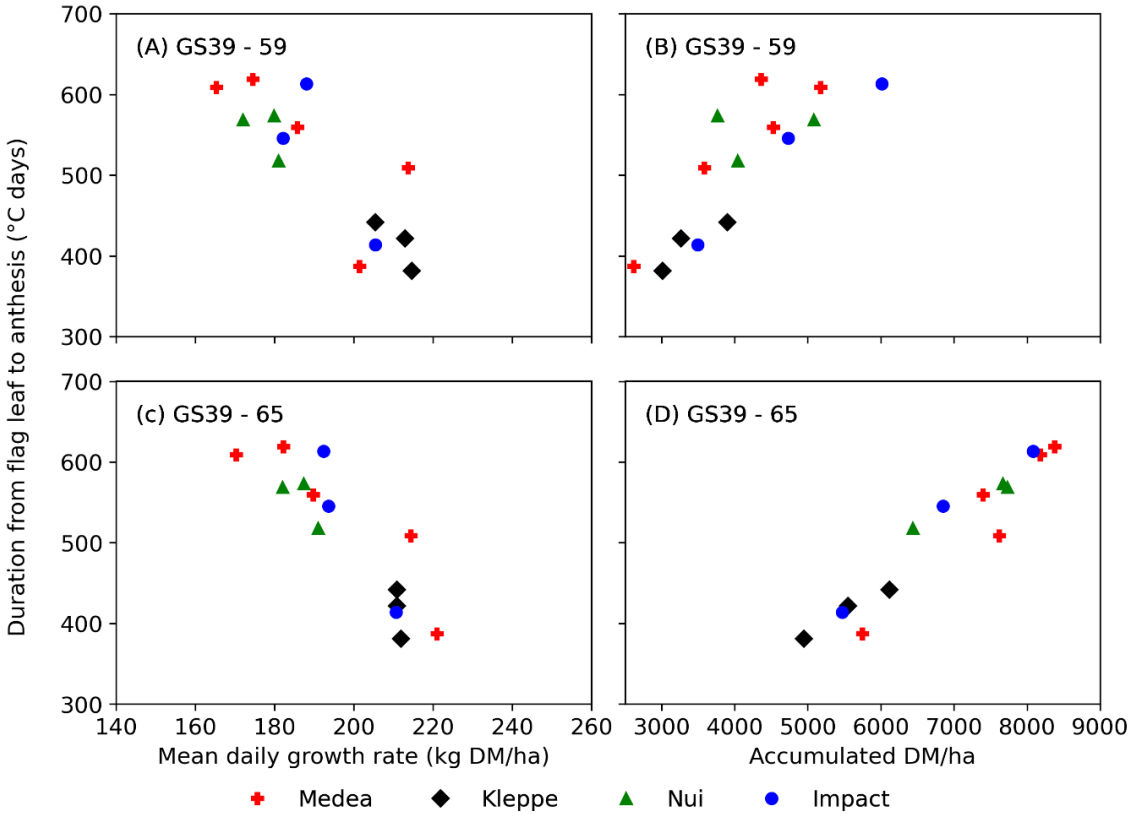


Figure 5.16. The duration from final leaf to flowering as influenced by calculated mean daily growth rates (A,C) and the accumulated dry matter (DM) production (B,D) of four perennial ryegrass genotypes sown of five dates at Lincoln, Canterbury, New Zealand in the 201920 growing season.

However, mechanisms for increased PDM and a reduced FL-AN are difficult to identify. The use of Tt to quantify the duration from FL-AN potentially removes the influence of temperature *per se*, unless the cardinal temperatures used in its calculation are inaccurate. However, the cardinal temperatures used are comparable to those of other species and those identified for other developmental stages in perennial ryegrass. Thus, leaving incident solar radiation and the resulting products from photosynthesis to reduce the duration of FL-AN. For the FL-AN duration to be shorter as a result of growth, the individual cells at the base of the stem would need to be genetically controlled and extend at an increased rate in response to radiation.

Therefore, the influence of Pp was investigated by using stem length and the number of spikelets/stem as indicators. It is possible that stem length was determined by the number of cells between nodes and that shorter stems would emerge faster during booting. Secondly, a reduction in the number of spikelets per stem would indicate less time for

differentiation and result in smaller seed head, which could take less time to emerge. Both hypotheses assume that the duration of cell division, which leads to potential stem length, is influenced by Pp, as Pp influences the duration between FI^{HS} and TS^{HS} (section 5.3.9). The assumption is that the FL-AN duration itself is a growth process that was fixed earlier in the crop lifecycle when the stem was initiated.

5.3.18 Reproductive stem length

For all genotypes at SD1-3, reproductive stem length was influenced (P=0.003) by a sowing date by genotype interaction. Stem length was constant for 'Kleppe' at 74.2 cm (±2.19) and 'Grasslands Nui' (69.2 cm) and reduced for SD3 for both 'Medea' and 'Grasslands Impact' (Figure 5.17, Appendix 14).

For 'Medea', across all five sowing dates, reproductive stem length reduced from 69.4 cm (±1.74) as sowing date was delayed. Stem length at SD3 (48.6 cm) and SD4 (45.4 cm) were the same while SD5 further reduced stem length to 38.1 cm (Figure 5.17).

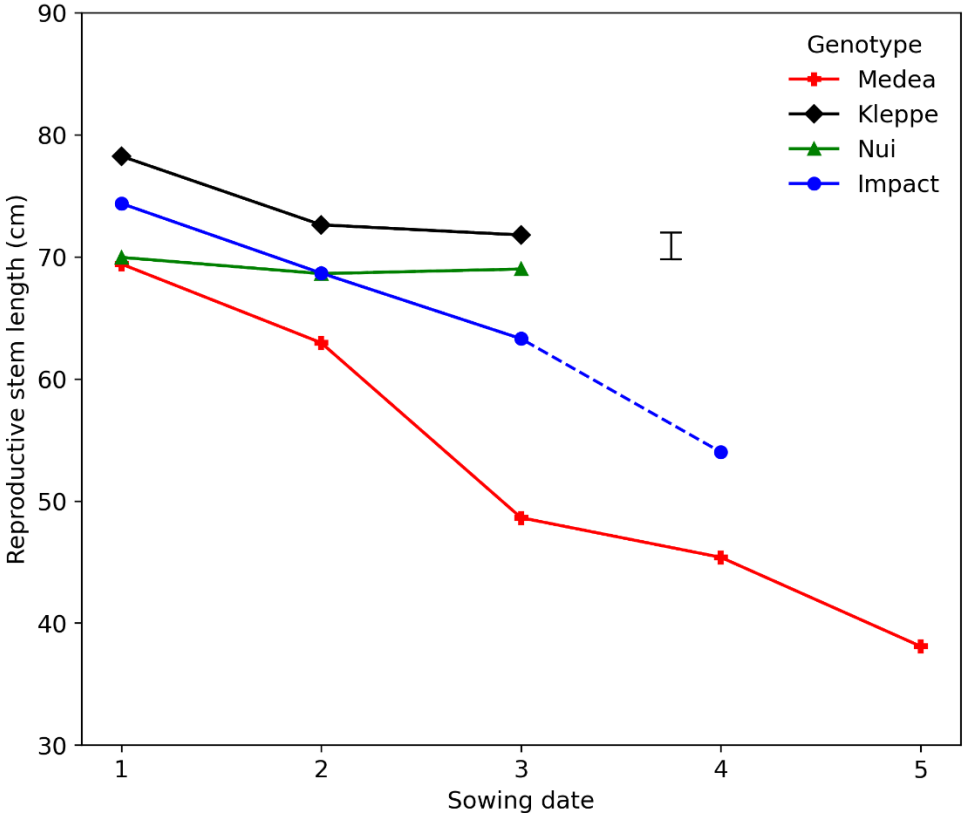


Figure 5.17. Reproductive stem length of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand. Bar is the interaction standard error of the mean (2.19). Dashed line is where less than 50% of plants achieved flowering.

Stem length was then plotted against the duration from FL-AN for individual genotypes. For 'Medea' the duration was not different for SD1 and SD2 (Table 5.16), and decreased with subsequent sowings. Fitting either a simple linear regression to data for SD3-5 or a split line regression to all sowing dates, resulted in a slope of 16.4 (± 0.67)°C days/cm of stem length reduction (Figure 5.18). The split line regression suggests the duration remains constant until a breakpoint at ~52 cm of stem length.

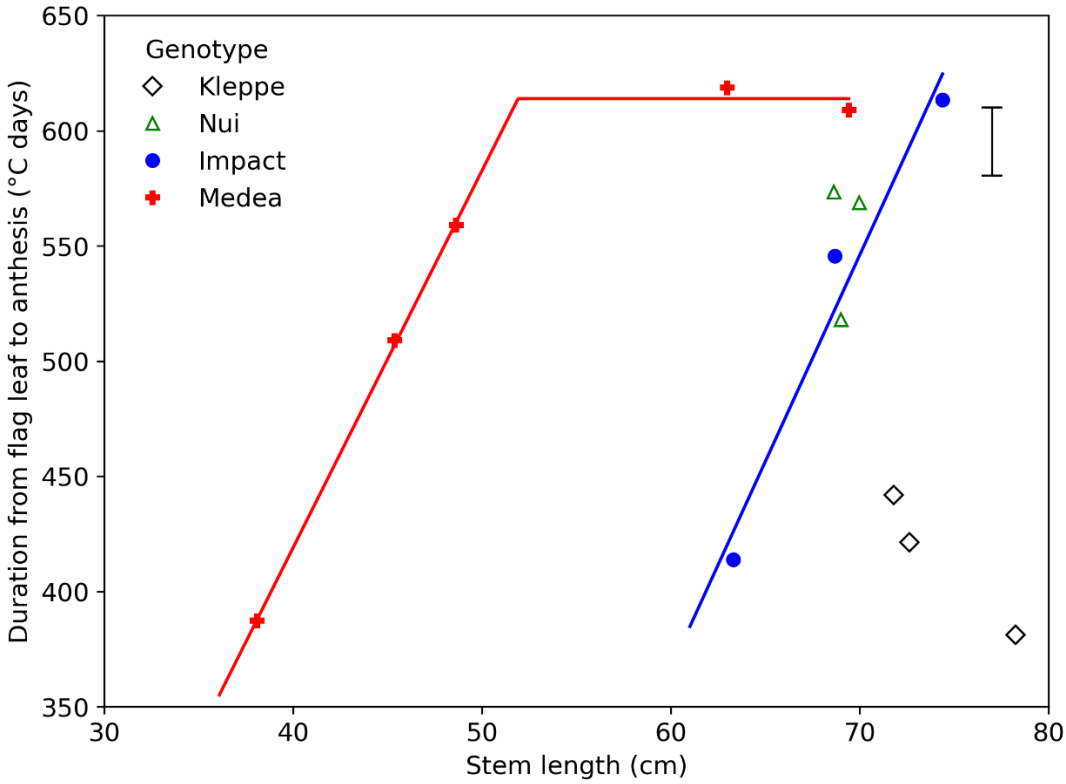


Figure 5.18. Duration from final leaf appearance to flowering (anthesis) (FL-AN) relative to reproductive stem length of four perennial ryegrass genotypes sown on five dates at Lincoln, Canterbury, New Zealand in the 2019/20 growing season. Regression fitted only to genotypes that showed a significant reduction in FL-AN via previous ANOVA, thus, 'Kleppe' and 'Grasslands Impact' are plotted for reference only. Bar = SEM for the genotype by sowing date interaction for FL-AN. 'Medea', FL-AN = breakpoint x = 51.9 (± 0.43), breakpoint y = 613.9 (± 3.62), slope of line 1 = 16.4 (± 0.67), R^2 = 0.99. 'Grassland Impact', FL-AN =17.9 (± 3.65) × stem length -709 (± 251), R^2 = 0.92.

For 'Grasslands Impact', a near linear response was shown with slope of 17.9 (± 3.65) °C days/cm of stem length reduction, consistent with that of 'Medea' (Figure 5.18). Next, stem length was considered as a possible result of the duration between FI and TS, when

the majority of cell division for building the stem occurs. For 'Medea' and 'Grasslands Impact', reproductive stem length reduced as the HS duration between FI and TS reduced (Figure 5.19). When a linear regression was fitted to both genotypes, it showed an intercept of 36 cm at a duration between FI and TS of 2HS. Stem length increased by 36.8 cm (±6.7) per HS as the duration between FI and TS increased (Figure 5.19). The stem length was consistent for 'Kleppe' and 'Grasslands Nui' (Appendix 15) even though the duration between FI and TS reduced with sowing date.

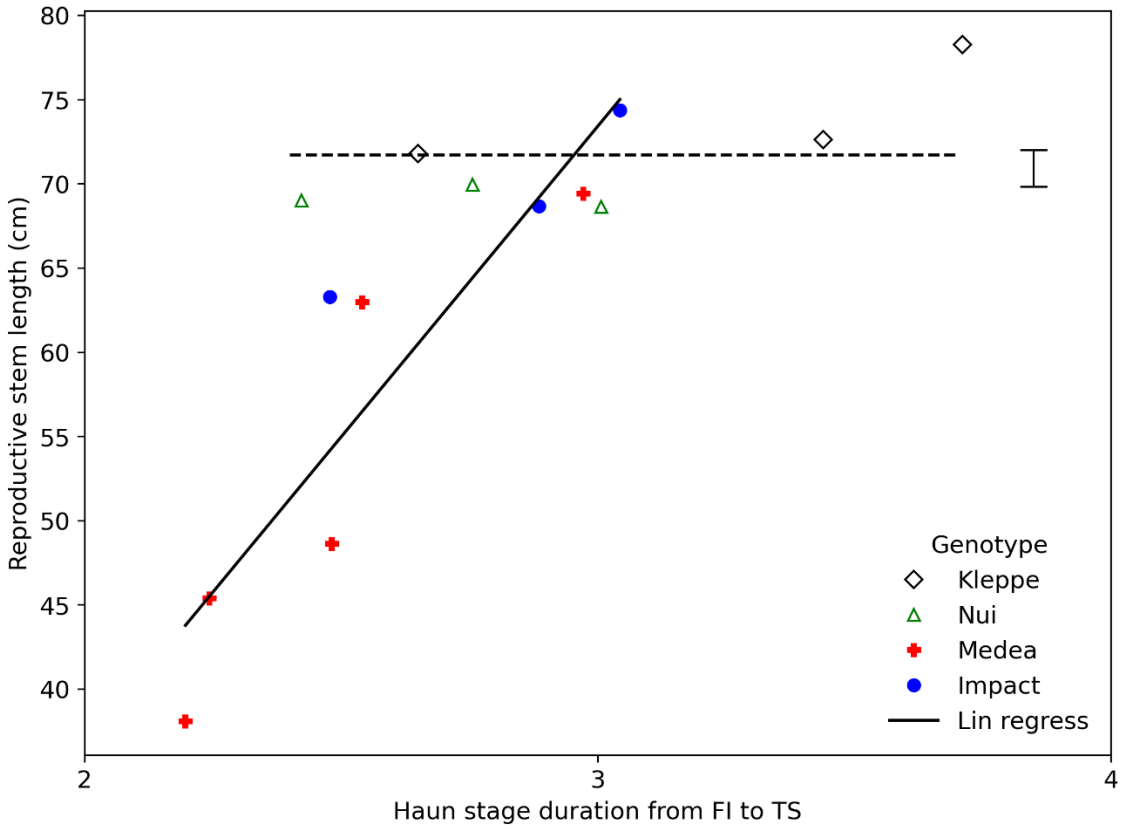


Figure 5.19. Reproductive stem length of four perennial ryegrass genotype as described by the Haun Stage (HS) duration between floral initiation (FI) and terminal spikelet (TS). Genotypes sown on five dates at Lincoln, New Zealand in the 2019/20 growing season. Bar is the SEM for stem length of the genotype by sowing date interaction. Regression is for 'Medea' and 'Grasslands Impact' only, where; stem length = 36.9 (± 6.7) × duration - 37.3 (± 17.5), R^2 = 0.83. 'Kleppe' and 'Grasslands Nui' plotted for reference, dashed line the mean of these two genotypes.

To confirm the relationship, the duration from FL-AN was plotted against the HS duration between FI and TS (Figure 5.20). For 'Medea', two straight lines described the relationship

where the duration of FL-AN reduced at 521 (± 173) °C days/HS, when the FI to TS duration was below 2.5 HS. For 'Grasslands Impact', a simple linear relationship explained the reduction in the duration from FL-AN by 347 (± 23.3) °C days per HS reduction in duration from FI to TS (Figure 5.20).

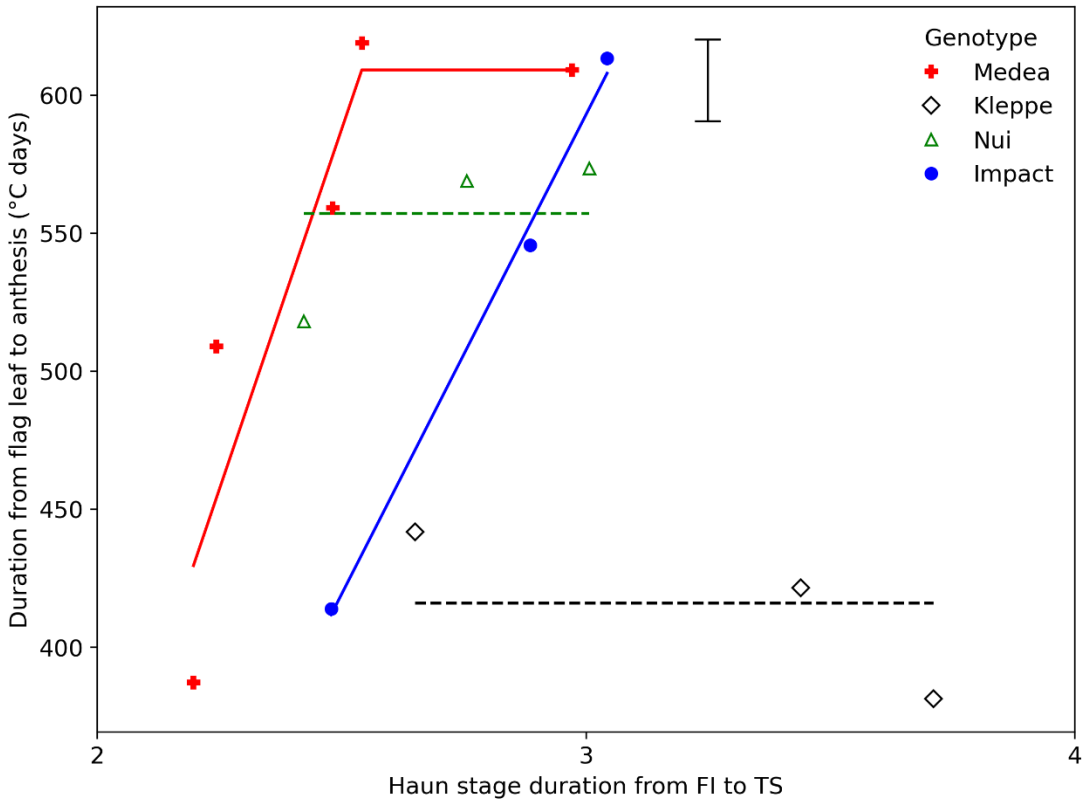


Figure 5.20. Duration from final leaf appearance to flowering (anthesis) (FL-AN) as relative to the duration from floral initiation (FI) to terminal spikelet (TS) of four perennial ryegrass genotypes sown on five dates at Lincoln, Canterbury, New Zealand in the 2019/20 growing season. Regression fitted only to genotypes that showed a significant reduction in FL-AN via previous ANOVA, thus, 'Kleppe' and 'Grasslands Impact' are plotted for reference only. Bar = SEM for the genotype by sowing date interaction for FL-AN. 'Medea', FL-AN = breakpoint x = 2.54 (± 0.12), breakpoint y = 609 (± 51.2), slope of line 1 = 521 (± 173), R^2 = 0.85. 'Grassland Impact', FL-AN =347 (± 23) × stem length - 449 (± 65), R^2 = 0.99.

5.3.19 Influence of Pp on the FL-AN duration

To determine the influence of Pp on the FL-AN duration for 'Medea' and 'Grasslands Impact', sowing date means of FL-AN were plotted against the mean Pp between FI and TS (Figure 5.21). For 'Medea', linear regression was fitted to the data for SD2-5 because the relationship between FL-AN and the duration between FI^{HS}-TS^{HS} occurred over this

range (Figure 5.20). Duration from FL-AN reduced from 620°C days by 45°C days/h Pp above 12.5 h.

For 'Grasslands Impact' the duration from FL-AN reduced from 620°C days by 76°C days/h Pp above 12 h (Table 5.18).

Therefore, FL-AN can be calculated by rearranging the regression equation, as per Equation 29, and reducing the duration from 620°C days as the mean Pp from FI-TS increases above ~12 h.

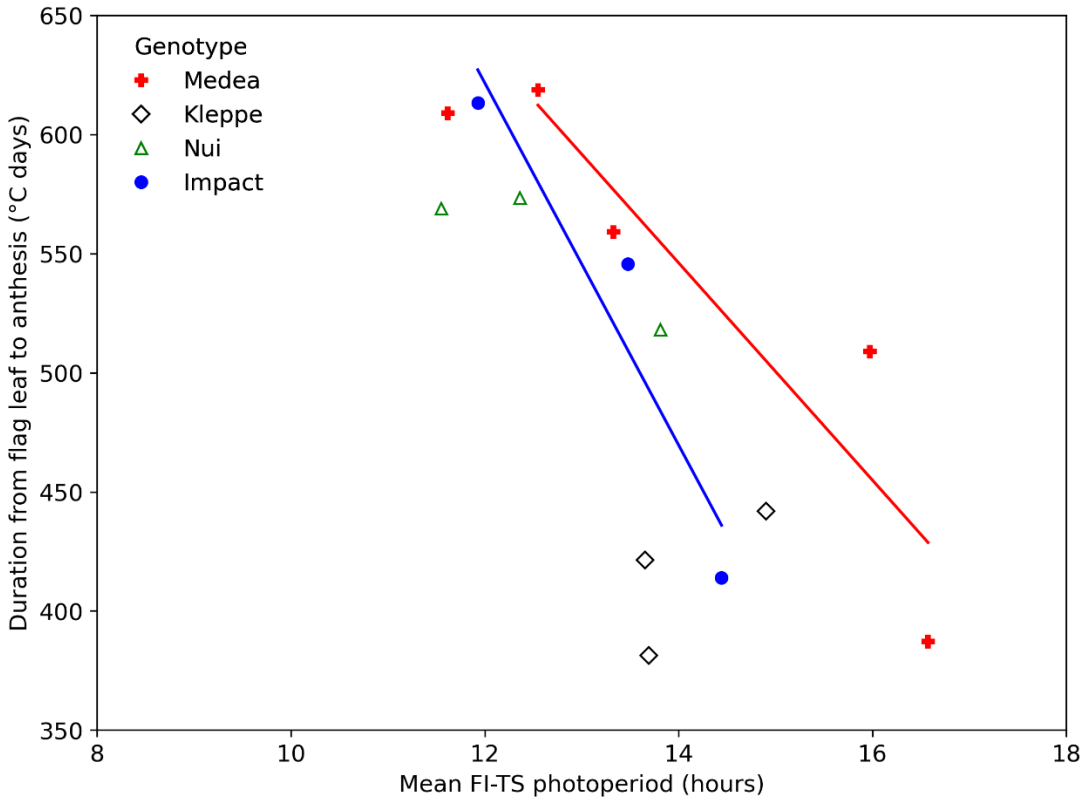


Figure 5.21 Thermal time duration, T_b =0°C, from final leaf fully emerged until flowering (anthesis) as influenced by mean photoperiod between floral initiation (FI) and terminal spikelet (TS). Data is the mean of three replicates from treatments that achieved 50% of plants flowering when sown on five sowing dates near Lincoln, New Zealand (-43.6° S latitude). Lines are linear regression for 'Grasslands Impact', y = -76 (±25) × FI-TSPp + 1533 (±330), R^2 = 0.90, and 'Medea' sowing dates 2-5, y = -45 (±14) × FI-TS Pp + 1185 (±212), R^2 = 0.90. 'Grasslands Nui' and 'Kleppe plotted for reference only.

Table 5.18. Parameters required to model the change in duration between final head emergence and flowering in response to the mean Pp between floral initiation and terminal spikelet of two perennial ryegrass genotypes.

Genotype	Pp_{crit} (h)	Tt at Pp_{crit} (°C days)	Pp_{max} (h)	Tt at Pp_{max} (°C days)	Slope (°C days/h Pp)
Medea	12.55	620	16.6	438	-45
Grasslands Impact	12	621	14.5	430	-76

$$
\begin{cases}
FL\text{-}AN = 620 & \text{If } FI\text{-}TS_{Pp} < Pp_{crit} \\
FL\text{-}AN = 620-(a*(FI\text{-}TS_{Pp} - Pp)) & \text{If } FI\text{-}TS_{Pp} > Pp_{crit} \text{ and } FL_{Pp} < Pp_{max} \\
FL\text{-}AN = 430 & \text{If } FL\text{-}AN < Pp_{max} \text{ (430°C days)}
\end{cases}
\qquad \text{Equation 29}
$$

Where:

620	is the maximum Tt from FL-AN
a	is the slope of the Pp regression (Equation 30)
Pp_{crit}	is the base Pp where FL-AN decreases
Pp_{max}	is the photoperiod where FL-AN Tt is minimised
430°C days	is the minimum Tt from FL-AN

Given that the duration from FI^{HS}-TS^{HS} was influenced by Pp (Section 5.3.9), it appears plausible that Pp could influence the duration from FL-AN in 'Medea' and 'Grasslands Impact' through influences imposed during the period between FI to TS, when the seed head is being differentiated. Subsequently the number of spikelets per stem was analysed relative to Pp between FI^{HS} to TS^{HS} (Appendix 15) and investigated relative to stem length.

5.3.20 Spikelet's per ear and stem length

Finally, the number of spikelets/ear was regressed against stem length. For genotypes 'Medea' and 'Grasslands Impact', where stem length and spikelets/ear were influenced by the duration from FI – TS (Appendix 15), stem length and spikelet number were closely related (R^2 = 0.98) as spikelet number increased by 0.41 (± 0.02) per cm of stem length (Figure 5.22). This supports the hypothesis that the Pp between FI and TS influenced both characteristics.

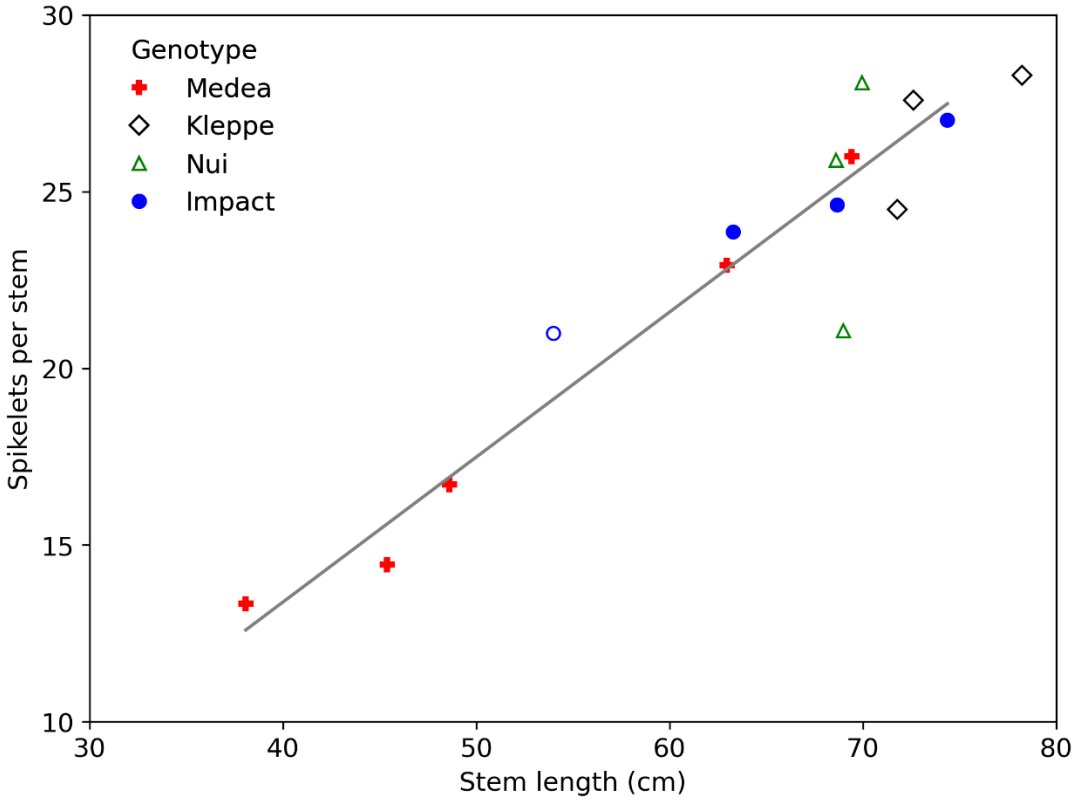


Figure 5.22. Spikelets per ear in relation to stem length of four perennial ryegrass genotypes sown on five dates at Lincoln, New Zealand in the 2019/21 growing season. Line is regression for Medea and Grasslands Impact only where, spikelets = 0.41 (± 0.02) × stem length -3.03 (± 1.34) (R^2 = 0.98). Open symbols are plotted for reference only.

5.3.20.1 Estimating FL-AN

Thus, FL-AN appears to be genetically controlled and was constant for 'Grasslands Nui' and 'Kleppe'. In contrast for 'Medea' and 'Grasslands Impact' FL-AN reduced as the HS duration from FI to TS decreased. For crop modelling, the duration of FL-AN requires confirmation from a minimum of two or three sowing dates where the HS duration from FI to TS are likely to be different. Where this is not possible, location of origin could be used to estimate the response. The origins of 'Grasslands Nui' and 'Kleppe' are from >45°N latitudes and these genotypes had a strong requirement for vernalisation. This compares with 'Medea' and 'Grasslands Impact' which originate from <45°N, and both had a weaker cold temperature response. Thus, based on these four genotypes, a break at approximately 48°N may provide an insight into this response.

5.3.21 Prediction of phase duration

Assuming V_{base} has been achieved (see Chapter 4) and the juvenile phase has passed, FI occurs when a genotype specific Pp_{base} is reached during lengthening Pp.

5.3.21.1 Influence of photoperiod on the number of leaves post floral initiation

Linear regression was fitted to the LN_{postFI} (Table 5.11) against the Pp at sowing, emergence, FL, DR, TS and final leaf ligule for all genotypes (Figure 5.23). LN_{postFI} effectively combines the ear develop (FI-TS) and stem extension (TS-FL) phases and when multiplied by the phyllochron produces a Tt target for FL predictions.

Saturation of the Pp response was found by substituting the minimum LN_{postFI}, of four leaves, to find x in each regression equation, parameters are presented in Appendix 16. The minimum LN_{postFI} of four leaves was selected as the mean of 'Medea' from SD3, 4 and 5 and was consistent with the minimum reported in Chapter 4 from controlled environment experiments.

For 'Medea' linear regression was fitted to SD1, 2 and 3 only as subsequent sowing dates had achieved Pp saturation at FI. The highest R^2 (0.86) and lowest slope P value were given by using the Pp at TS where the slope was -0.89 leaves per hour of Pp increase with a saturating Pp of 14.1 hours (Appendix 16).

For 'Kleppe', the Pp at TS showed an R^2 = 0.99 and a P value of 0.01 when describing the LN_{postFI} in response to Pp. The slope of the relationship was -4.67 leaves per hour of Pp and a saturating Pp of 15.5 h calculated (Figure 5.23E).

For 'Grasslands Nui', the slope of the relationship was –0.66 leaves per hour of Pp and a saturating Pp of 15.6 hours calculated when using the Pp at TS (Appendix 16).

For 'Grasslands Impact' the slope of the relationship was –0.55 leaves per hour of Pp and a saturating Pp of 17.13 hours using the Pp at TS (R^2 = 1.0, P = 0.01) (Appendix 16).

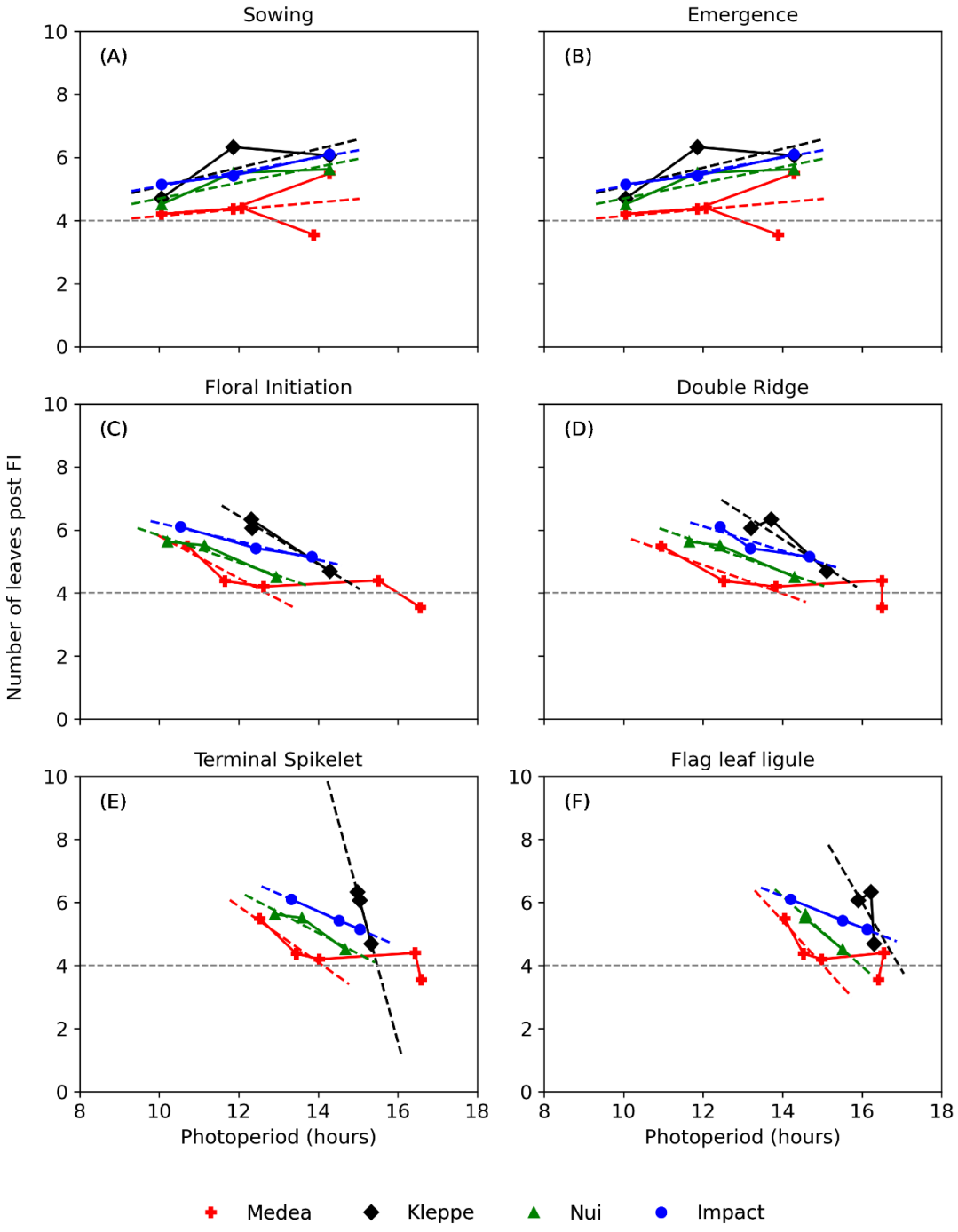


Figure 5.23. Number of leaves produced post floral initiation (FI) in relation to the photoperiod (Pp) at eight different event timings of four genotypes of perennial ryegrass sown of five dates near Lincoln, Canterbury, New Zealand. Dashed lines represent linear regression for data presented, except for 'Medea' in C,D,E and F where regression represents the first three sowing dates only. Grey dashed line is constant four leaves which represents the minimum possible at Pp saturation. Data only presented if more than 50% of plants flowered for a specific sowing date.

5.3.21.2 Predicting number of leaves post floral initiation.

The slope of this relationship was steep and a function is required to limit LN_{postFI} to a genotype maximum of 5 for 'Medea' and 6.3 for all other genotypes. Additionally, the minimum LN_{postFI} is 4.0 when FI occurs at, or beyond, Pp_{sat}. Thus, the number of leaves remaining to emerge ranges from 6.3 at FI in short Pp to a minimum of 4.0 and can be calculated by Equation 31.

$$LN_{postFI} = LN_{min} + Pp_{slope} \times (Pp_{sat} - Pp) \quad \text{Equation 31}$$

Where:

LN_{min}	is the minimum leaf number possible at Pp_{sat}, equal to 4
Pp_{slope}	is the slope of the Pp response
Pp_{sat}	is the Pp where leaf number is 4
Pp	is the photoperiod at FI

If:

$LN_{postFI} < 4$, $LN_{postFI} = 4$ and if $LN_{postFI} > 6.3$, $LN_{postFI} = 6.3$

Calculations made utilising the mean slope and saturating Pp between FI and TS (Appendix 16) showed a strong relationship between predicted and observed data (Figure 5.24). For example, the predicted LN_{postFI} = 0.999 (±0.010) x observed LN_{postFI} (R^2 = 0.99, P<0.001).

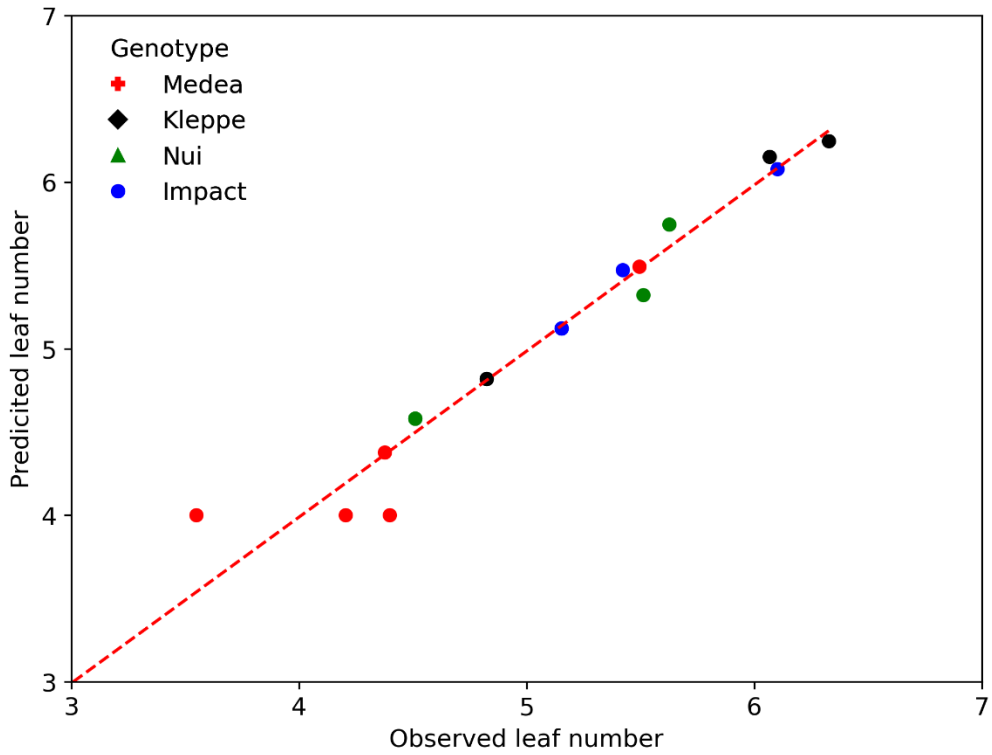


Figure 5.24. Number of predicted main stem leaves compared with observed main stem leaves, calculated using Equation 31 by substituting parameters at terminal spikelet from Appendix 16 for four perennial ryegrass genotypes sown on five date near Lincoln, New Zealand in the 2019/20 growing season. Line = regression analysis constrained to the origin where; predicted = 0.999 × observed, R^2 = 0.99.

5.3.21.3 Predicting final leaf emergence date.

The date of final leaf appearance can be calculated by multiplying the number of leaves remaining to appear at FI, by the phyllochron (Table 5.6) to determine a Tt target. Subsequently, the actual in field measured Tt was accumulated to calculate the day of final leaf emergence. FI was restricted to when plants had four leaves, when the vernalisation function was saturated and the current Pp exceeded the Pp_{base}. For spring sown 'Medea', the Tt target for FL emergence was calculated as 1268°C days by:

- 188°C days for emergence +
- (5 HS prior to FI (Table 5.7) + 4 HS post FI (Figure 5.23)) ×
- the mean phyllochron of 120°C days (Table 5.6).

Across all data, mean final leaf emergence was consistent with predicted values, but ranged from 72°C days early for 'Medea', SD2, to 74°C days late for 'Grasslands Nui' in

SD3. In days of actual temperature experienced, this ranged from ±6 days for 'Medea' to +6 days for 'Grasslands Impact' for in SD1 (Table 5.19).

Table 5.19. Predicted and observed dates of final leaf emergence and the thermal time (Tt, °C days) from predicted floral initiation (FI) until final leaf emergence of four perennial ryegrass genotypes sown on three dates near Lincoln, New Zealand.

Genotype	Sow date	FI (doy)	Pred Tt	Obs Tt	±	Predicted date	Observed date	±
Medea	1. 1/03/2019	200	661	620	41	10/10/2019	7/10/2019	3
	2. 17/04/2019	200	661	733	-72	10/10/2019	16/10/2019	-6
	3. 14/06/2019	257	494	420	74	31/10/2019	25/10/2019	6
	4. 30/8/2019	306	1268	1264	35	13/12/2019	11/12/2019	2
	5. 4/10/2019	326	1268	1248	17	1/01/2020	31/12/2019	1
Kleppe	1. 1/03/2019	251	841	880	-40	21/11/2019	24/11/2019	-3
	2. 17/04/2019	251	841	865	-24	21/11/2019	23/11/2019	-2
	3. 14/06/2019	259	841	908	-67	25/11/2019	29/11/2019	-4
Nui	1. 1/03/2019	200	802	744	58	22/10/2019	17/10/2019	5
	2. 17/04/2019	204	795	766	28	25/10/2019	22/10/2019	3
	3. 14/06/2019	257	639	562	77	9/11/2019	5/11/2019	4
Impact	1. 1/03/2019	210	825	754	71	1/11/2019	26/10/2019	6
	2. 17/04/2019	210	825	886	-60	1/11/2019	5/11/2019	-4
	3. 14/06/2019	258	748	774	-26	18/11/2019	20/11/2019	-2
	Average	242	822	816	8	10/11/2019	9/11/2019	-1

When plotted as predicted and observed dates of final leaf emergence, the relationship was described via linear regression as; predicted = 1 × observed (R^2 = 0.97) when the origin is constrained to zero (Figure 5.25).

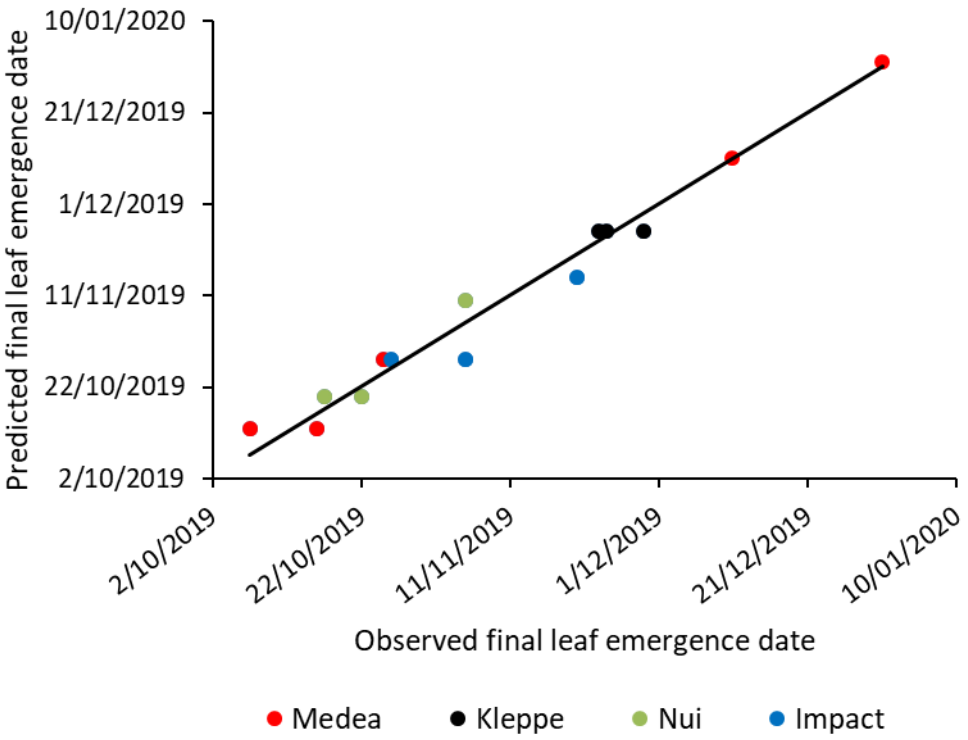


Figure 5.25 Predicted and observed dates of final leaf emergence of four perennial ryegrass genotypes sown on three dates near Lincoln, New Zealand. Line from regression where; predicted = 1 × observed. (R^2 = 0.97).

5.3.21.4 Predicting final leaf emergence using the ARCWHEAT approach

In this approach, zero progress towards flowering is made unless both V_{base} and Pp_{base} are reached. Pp_{base} was estimated as the shortest Pp from the calculated FI dates for each genotype. The Pp_{base} was set to 10.5 h for both 'Medea' and 'Grasslands Nui', 10.7 h for 'Grasslands Impact' and 12 h for 'Kleppe' based on the Pp requirements demonstrated for FI between SD1 and SD2. Saturating Pp was calculated as per Section 5.3.21 at four developmental timings (Appendix 16, Figure 5.26). The saturating Pps, calculated for DR and TS were consistent, effectively the mean of these events set Pp_{sat}. The Pp at final leaf emergence did not separate these genotypes and thus is unsuitable for modelling (Figure 5.26D).

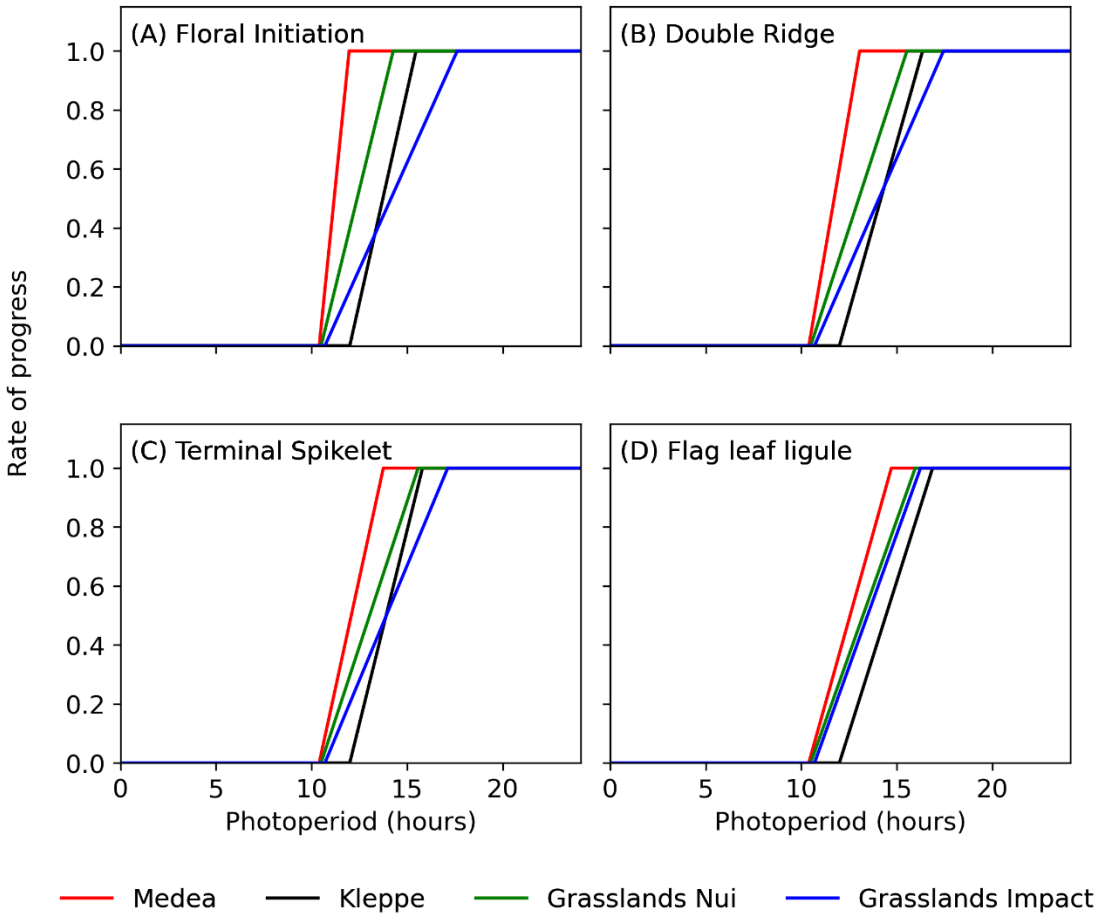


Figure 5.26. Potential photoperiod (Pp) models for four genotypes of perennial ryegrass based on Pp at four developmental timings.

FI was determined when the vernalisation function developed in Chapter 4 was saturated and the current Pp exceeded the Pp_{base}. By setting genotype specific parameters and accumulation targets (Table 5.20), daily Tt values were multiplied by a Pp factor and summed from predicted FI date. When Tt targets were achieved, FL appearance date was calculated. Across all data, predicted mean final leaf emergence followed the pattern of observed values where the variance ranged from ±6 days (Figure 5.27, Table 5.20).

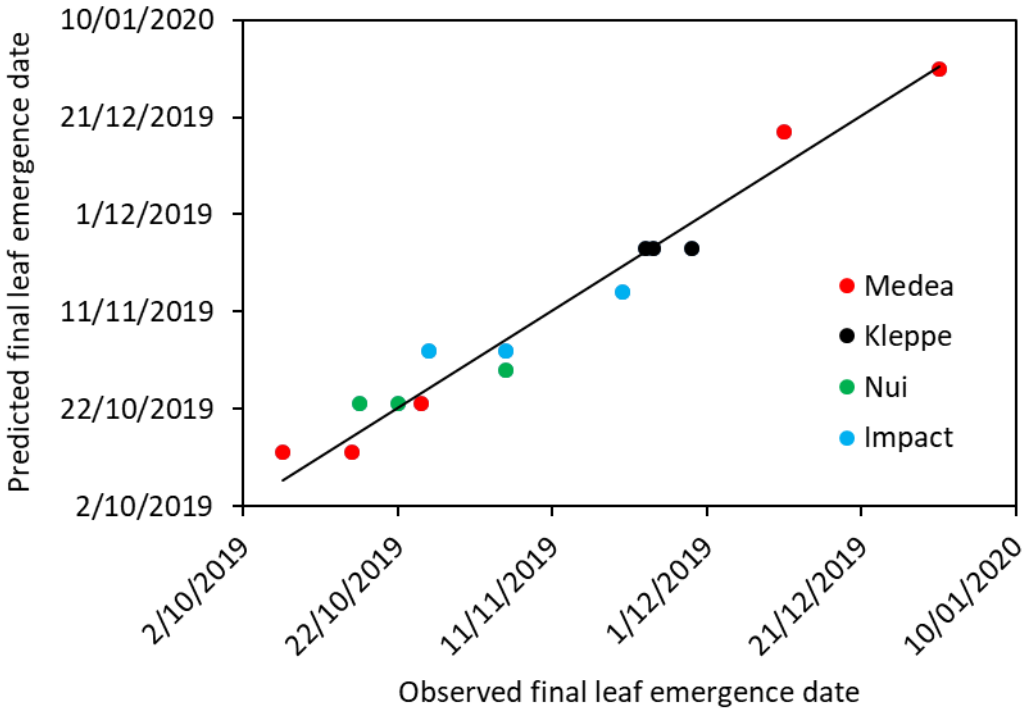


Figure 5.27. Observed and predicted dates of final leaf emergence using modified thermal time of four perennial ryegrass genotypes sown on three dates near Lincoln, New Zealand. Line from regression where; predicted = 1 × observed. (R^2 = 0.96).

Table 5.20. Key parameters for modelling via the modified thermal time approach along with predicted and observed dates of final leaf emergence of four perennial ryegrass genotypes sown on three dates near Lincoln, New Zealand.

Genotype	Sow date	Pp_{base}	Saturating Pp	Tt target	Predicted date	Observed date	±
Medea	1. 1/03/2019	10.5	14	375	10/10/2019	7/10/2019	3
	2. 17/04/2019				10/10/2019	16/10/2019	-6
	3. 14/06/2019				31/10/2019	25/10/2019	6
	4. 30/8/2019				13/12/2019	11/12/2019	2
	5. 4/10/2019				1/01/2020	31/12/2019	1
Kleppe	1. 1/03/2019	12	15.8	620	21/11/2019	24/11/2019	-3
	2. 17/04/2019				21/11/2019	23/11/2019	-2
	3. 14/06/2019				25/11/2019	29/11/2019	-4
Nui	1. 1/03/2019	10.5	15.6	350	22/10/2019	17/10/2019	5
	2. 17/04/2019				25/10/2019	22/10/2019	3
	3. 14/06/2019				9/11/2019	5/11/2019	4
Impact	1. 1/03/2019	10.7	17.5	325	1/11/2019	26/10/2019	6
	2. 17/04/2019				1/11/2019	5/11/2019	-4
	3. 14/06/2019				18/11/2019	20/11/2019	-2
	Average				9/11/2019	10/11/2019	0.3

5.1 Discussion

The objective of this chapter was to quantify the timing of phenophases from emergence to flowering under field conditions with natural changes in photoperiod. This was achieved through a field experiment utilizing four perennial ryegrass genotypes sown on five dates that combined to produce a range of phenological responses. Based on differences that occurred in the timing of FI and flowering, the null hypothesis was rejected.

5.1.1 Sowing to emergence

Emergence was conservative between genotypes at 188°C days when soil temperature recorded at 3 cm depth was used to calculate Tt (T_b, 0°C) (Figure 5.4, Figure 5.5). This is longer than the 133°C days presented by Moot *et al.* (2000) for field emergence of 'Grasslands Nui' with a T_b of 2.1°C. In wheat, 150°C days is used by *Sirius* and ARCWHEAT1 (Weir *et al.* 1984; Jamieson *et al.* 1998b) based on air temperature, as soil temperature at seed depth is not commonly collected. When using air temperature, sowing date influenced the duration to emergence which was longer in winter than summer, comparable to using days after sowing to describe emergence (Figure 5.4). These results indicate that soil temperature at seed depth was different from air temperature, particularly in winter. Therefore, soil temperature provided a more accurate estimate of the duration to emergence and should be used for model development (Jamieson *et al.* 1995b). Thus, if soil temperature is not available, it is suggested that 200°C days calculated from air temperature be used to estimate emergence until further information is gathered.

5.1.2 Flowering including vernalisation requirements

For flowering, the genotype 'Medea' acted as a 'long day' plant and flowered over summer from all sowing dates (Figure 5.6). Genotypes 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact' achieved more than 50% of plants flowering from sowings prior to mid-winter. However, in an increasing photoperiod, the percentage of plants that flowered reduced for all genotypes. The reduction was most noticeable in 'Kleppe', with no flowering from SD4 and SD5. These results are similar to those of Cooper (1959b) (two cultivars) and Aitken (1966) (four cultivars) who showed a reduction from ~100% plants

flowering for autumn sowing to <10% when sowing was until delayed after mid-winter at Aberystwyth, Scotland and Melbourne, Australia respectively. Additionally, both studies demonstrated a delayed ear emergence date as sowing approached mid-winter, which was also found for 'Medea' and 'Kleppe' (Table 5.14). However, there are differences among and within genotypes. For example, 'Grasslands Impact' showed a gradual decline in the percentage of plants heading as sowing was delayed into spring, similar to the 'Irish perennial ryegrass' presented by Cooper (1959b). In contrast, no 'Kleppe' plants flowered from spring sowings, similar to genotype 'Kent' (Cooper 1959b). This demonstrates within genotype variation and is not unexpected given the outcrossing nature of perennial ryegrass.

These results support the hypothesis proposed in Chapter 4, that perennial ryegrass required a dual induction with exposure to cool temperatures, and/or short photoperiod before separate long Pps complete SI. Utilising the vernalisation model from Chapter 4, Section 4.3.12, 'Medea' was the only genotype to achieve V_{base} from spring sowing, and thus the only genotype to flower. The requirement of PI to occur before flowering can proceed, effectively provides an on/off switch for flowering in perennial ryegrass.

5.1.3 Final leaf emergence and flowering dates

Date of final leaf emergence and flowering were influenced primarily by genotype where within a sowing date, the pattern was consistently; 'Medea' followed by 'Grasslands Nui', 'Grasslands Impact' and lastly, 'Kleppe'. These results show the observed differences among genotypes are genetically controlled (Cooper 1952, 1956b, 1959a; Beddows 1968; Camlin 1975; Abel *et al.* 2017) (Table 5.13). Generally, delayed sowing corresponded to a delay in FL emergence (Table 5.13), most evident in 'Medea', where SD1 reached FL appearance four days after the sowing of SD5, and 'Grasslands Impact', where final FL differed by 24 days between SD1 and SD3. Changes in the date of FL emergence and flowering from different sowing dates mean the simple Tt models that accumulate from mid-winter, as proposed by Beddows (1968), Hurley *et al.* (2006), Abel *et al.* (2017) etc, are missing key phenological information to accurately estimate developmental timings as sowing dates move towards the shortest day in a New Zealand environment.

5.1.4 Number of main stem leaves

The number of main stem leaves (FLN) was reduced as sowing was delayed from autumn to winter in all genotypes (Figure 5.8). The production of additional leaves, from earlier sowings, is a delaying mechanism to synchronise flowering with the summer months following winter. For example, for autumn sowings the FLN reduced for all genotypes as sowing was delayed. For 'Medea', SD1 produced ~16 main stem leaves which reduced to the lowest possible number of ~9 from winter and spring sowing. For 'Kleppe', Grasslands Nui' and 'Grasslands Impact' the FLN was lowest from winter sowings as incomplete vernalisation limited flowering from spring sowings. This pattern is consistent with other monocot species that achieve FI in response to vernalisation or lengthening Pp e.g. oats (Sonego 2000), wheat (Brooking & Jamieson 2002) and barley (Kirby *et al.* 1985). In all cases the final number of leaves maybe more than 15 for autumn sowing but reduced towards 7 for winter sowings.

FLN followed the expected pattern (Figure 5.1) where for autumn sowings FLN was negatively correlated to lengthening Pp (Figure 5.15) and the Pp response between FI and TS described the response (Table 5.12). The use of the Pp at TS was supported by visual assessments where at TS the apex had differentiated all main primordium into either leaves or spikelets. At this point the FLN cannot be further adjusted. Until TS, primordium at the base of the developing ear cannot be confirmed as leaves or spiklelets, supporting the hypothesis that the FLN is set around the time of TS. However, it is plausible that TS and FLN are set at some stage earlier and it takes some time for the response to become visible on the apex. Confirmation of this would require identification and quantification of genes that control these processes. The use of Pp at TS for estimation of FLN is supported by Griffiths *et al.* (1985) for wheat and Sonego (2000) for oats, who both suggest photoperiod sensitivity can continue until TS formation. In contrast, Brooking *et al.* (1995) suggested that there is some genetic variation between genotypes of spring wheat, but supported the concept that FLN is committed between FI and TS. Thus, until further work can be completed with extra autumn and winter sowing dates to add additional data points, the mean of the FI and TS responses appears adequate to make predictions from.

5.1.5 Phyllochron

A two line model beginning at 188°C days, the duration to emergence, described the relationship between leaf appearance and Tt (where the inverse of each slope parameter is the phyllochron). Prior to ~9 leaves, the phyllochron was ~115°C days, following which phyllochron increased to ~150°C days (Figure 5.7). These are similar to the phyllochron shown in Chapters 3 and 4. The initial phyllochron was similar to that reported for perennial ryegrass at 96 - 110°C days (Davies & Thomas 1983; Bahmani *et al.* 2000; Black *et al.* 2002) while the subsequent section is similar to the 167°C days used by Romera *et al.* (2009). The reduction in phyllochron post ~HS8 was associated with an increase in the length of the leaf blade as discussed in Chapter 3. These results produce a model similar to that of Brown *et al.* (2013) and He *et al.* (2012) to describe leaf appearance rate in wheat. Thus, the use of HS accounts for temperature variation and allows direct comparison among treatments and genotypes. For phyllochron calculations, 3 cm soil temperature was used until FI followed by canopy temperature once stem extension was observed. These were considered preferable to air temperature as the plant perceives temperature at the stem apex which is located at or below soil level prior to FI. Therefore, if the appropriate temperatures are not recorded they should be estimated.

5.1.6 Pattern of primordium production

Vegetative plants produced primordium at a rate of 2.25/HS up until HS8, followed by a reduction to ~1/HS (Figure 5.9). Thus, at and above HS8 there are 12 organs to emerge from the leaf sheath ((HS8 × 2.25 + 2 = 20) - HS8 = 12). This is a similar pattern to that identified in Chapter 4, Section 4.3.6.1 and discussed in Section 4.4.7. This confirms the pattern of primordium production under both field and controlled environment chamber conditions for plants that do not achieve FI.

For reproductive plants, primordium production followed the expected pattern, as seen in Chapter 4, and was described using three straight lines (Figure 5.10). The rate of primordium production during vegetative growth approximated the early phase of the 'vegetative' model at 2.21(±0.06)/HS and was not different to the 2.17(±0.04) and 1.97(±0.04)/HS identified in Section 4.3.6. This confirms that ~2 primordium are produced per HS under both field and controlled environment conditions across a range of temperature and Pp conditions, until either ~HS8, or FI. This pattern mimics wheat and

oats where prior to FI, primordium production is constant at ~2 (Jamieson *et al.* 1998b) and 1.7/HS (Sonego 2000), respectively. Thus, a sensible approach is to assume two primordium are produced prior to either ~HS8 (median of Chapters 4 and 5), and subsequently the plastochron equals the leaf appearance rate, or FI occurs.

5.1.7 What determines FI

FI occurred at a different HS for each genotype by sowing date combination (Table 5.7). This confirms that leaf stage does not determine the onset of FI. The FI^{HS} decreased as sowing date was delayed indicating that the mechanism for delaying FI is to produce leaves until the PI, SI and ear development phases are complete. This is not different from the results in Chapter 4 where FI^{HS} increased as the duration of vernalisation increased under 8 h Pp, and delayed the onset of FI (Figure 4.5), even following obtainment of V_{sat} (Section 4.3.6.2). Thus, production of leaves increases the FLN associated with an increase in HS during vernalisation. Further support is provided by SD1, where all genotypes reached V_{sat} sometime prior to the FI (Table 5.5). Thus, it becomes apparent that vernalisation alone did not induce FI. The timing of FI was determined by genotype specific Pp following V_{sat}. These results support an obligate requirement of long, or at least lengthening, Pp for FI, as proposed by Cooper (1960) and Evans (1960) and supported by the review of Heide (1994). This contrasts wheat, where FI was obtained under 8 h Pp treatments and occurs in association with V_{sat} (Robertson *et al.* 1996; Brown *et al.* 2013). In ARCWHEAT1, Pp_{base} is set to 0 h until DR supporting the proposal that in wheat, DR will occur regardless of Pp if the vernalisation function allows development to progress (Weir *et al.* 1984).

The values of Pp_{base} proposed are similar for 'Medea' 'Grasslands Nui', and 'Grasslands Impact' ranging from 10.4 – 10.8 h, while 'Kleppe' is longer at ~12 h (Figure 5.23). These are substantially shorter than the 'critical' Pp published for many perennial ryegrass genotypes, particularly those from controlled chamber/lighting experiments. For example, Aamlid *et al.* (2000) showed no flowering in the genotype 'Kleppe' below 16 h and suggested a critical Pp of 17.5 h to achieve flowering when grown under controlled environment conditions. At, Lincoln, New Zealand, the maximum Pp is 16.6 h, thus, if the Pp suggested by Aamlid *et al.* (2000) was correct, 'Kleppe' would not have flowered in this

experiment. Aamlid *et al.* (2000) suggested a critical Pp for other genotypes ranged from 12-14 hours depending on geographic location of origin, and Heide (1994) suggested a 9-10 h Pp was required for Mediterranean genotypes while up to 16 h may be required for those from higher latitudes. Conversely, Hurley *et al.* (2006) showed critical photoperiods of between 12.7 and 15 h for eight 'late season' perennial ryegrass genotypes when grown outdoors at Crossnacreevy in Northern Ireland. Equally, Keatinge *et al.* (1979) reported DR occurred at between 11.7 – 15.3 h for four perennial ryegrass genotypes at the same location. In the studies by Keatinge *et al.* (1979) and Hurley *et al.* (2006), the apex was exposed and the critical Pp identified as the time when DR was first observed, which is approximately 1HS later than the calculated FI in this experiment.

From the discussion above, it appears the direction of Pp change may be an important factor influencing the Pp_{base}. When Pp_{base} or 'critical photoperiod' was identified in field conditions here, and by Keatinge *et al.* (1979) and Hurley *et al.* (2006), Pp_{base} was considerably shorter than those presented by Aamlid *et al.* (2000) and those seen in Experiments 1–4 for 'Medea'. For example, when 'Medea' was sown in March, V_{sat} was achieved on 18/4/2019 in a decreasing Pp of 11.8 h. However, FI was not recorded until early spring at an increasing Pp of 10.7 h, less than the 11 h Pp were no 'Medea' plants flowered in Experiment 3, Section 3.3.2. This supports results presented by Cooper (1950) who showed FI was a result of increasing photoperiod in the spring. These results suggest perennial ryegrass is very sensitive to the change in Pp direction. For example, the Pp on the shortest day at Lincoln, New Zealand, is ~10.0 h and FI occurred between 10.4 and 10.8 h in sowing date one for, 'Medea', 'Grasslands Nui' and 'Grasslands Impact'. Therefore, to allow accurate estimation of FI, a field derived genotype specific Pp_{base} is required.

These data support the hypothesis that perennial ryegrass has a juvenile phase in relation to completing SI where no treatment achieved FI before HS4 and more commonly HS5 (Table 5.7). This is consistent with data from the controlled environment chambers and combined show a minimum of four leaves are required for FI. For example, in SD3 (mid-winter) FI occurred on 10/9/2019 for 'Medea', at HS4.25, while V_{sat} was achieved on 8/8/2019. The Pp at V_{sat} was 11.1 h, longer than the Pp_{base} identified in SD1, but FI was

delayed for an additional month (Table 5.7). Calculated HS4 (188°C days + 4 phyllochron) occurred 16/9/2019, approximately 6 days later than the observed HS4. At HS4, a total of 10 primordium have been committed and are likely to form leaves, particularly if FLN is committed at FI. FLN was 8.5 suggesting that FI occurred between HS3 and HS4, with a lag before becoming visible. Additional support is shown in SD3 where 'Grasslands Nui' and 'Grasslands Impact' produced 9.66 and 10.5 main stem leaves respectively after achieving FI at ~HS5.25, similar to that of 'Medea' from SD4 and 5. Thus, since perennial ryegrass can be vernalised as germinating seeds and seedlings (Cooper 1960), there is no juvenile phase for PI. Therefore, SI is limited via Pp perception where the requirement of 4-5 leaves provides the likely mechanism required for delaying FI. No examples were found in the literature to suggest a juvenile phase for perennial ryegrass, but most work has been completed on plants with more than four leaves at the end of vernalisation. However, in other perennial grasses e.g. cocksfoot (*Dactylis glomerata*), the juvenile phase has been shown to be related to leaf area and potentially carbohydrate supply (Calder 1963, 1964; Heide 1987), potentially a similar mechanism is present in perennial ryegrass.

5.1.8 Modelling FI

Modelling FI assumes the apex can respond to Pp as soon as V_{base} is reached (Chapter 4) and FI occurs on the day the Pp_{base} is reached in lengthening Pp. FI cannot occur until HS4, calculated from emergence using phyllochron and soil temperature data. Following ~660°C days and the obtainment of V_{sat}, FI occurs when the Pp is increasing and the genotype specific Pp_{base} is reached.

5.1.9 FI-TS

The duration between FI^{HS} and TS^{HS} decreased as sowing was delayed (Table 5.8) and as FI occurred in longer Pp. The relationship was described as $TS^{HS} = FI^{HS} \times 1.12+1.86$ (Figure 5.12) suggesting the shortest duration from FI^{HS}-TS^{HS} is ~1.86 HS that increased by 0.12 as the FI^{HS} increased. This confirms the shortest duration of ~2HS identified in Chapter 4 and that found in wheat by Brown *et al.* (2013) when FI occurred in 16 h Pp. Thus, the mechanism that extends the duration between FI^{HS} and TS^{HS} is short Pp (Figure 5.13). Extending the HS duration between FI^{HS} and TS^{HS} allows for further primordium production, some of which form leaves and thereby delay ear emergence through an

increase in the number of leaves left to emerge after FI. This is supported by Brown *et al.* (2013) who demonstrated the duration from FI-TS was extended by exposure to an 8 h Pp in wheat, compared with 16 h Pp. In essence, this response is the same as that used in both *Sirius* and ARCWHEAT1 where short spring days reduce the rate of progress towards flowering.

5.1.10 TS-final leaf

The calculated HS duration from TS^{HS} to final leaf emergence was conservative among treatments for Kleppe', 'Grasslands Nui' and 'Grasslands Impact' with a mean 2.6 HS, while Medea' was slightly shorter at ~2HS (Table 5.10). Regression analysis identified a constant 2.06 HS for all 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact' treatments with slope values of close to 1 (Figure 5.14). This can be considered the same as the FLN = $TS^{HS}\times0.98+2.09$ (Figure 4.16) that was identified in Chapter 4, and discussed in Section 4.4.8. Thus, ~2 leaves are left to appear after the TS stage is reached (mean of the three data sets) and FLN can be estimated by FLN = $TS^{HS}+2$. This relationship also aligns with wheat, for which Brown *et al.* (2013) used FLN = $2.86+1.1\times TS^{HS}$ to describe the duration between TS^{HS} and FLN. The regression intercept in this study for 'Medea' was 0.89, which was lower than the 2HS shown in Chapter 4. This result potentially indicates genotype differences when Pp is lengthening and requires further investigation. Therefore, it is hypothesised that Pp does not affect the duration between TS and final leaf emergence.

5.1.11 Duration between FI, TS and final leaf appearance

The minimum possible number of leaves produced post FI (LN_{postFI}) was ~4, by 'Medea' when sown during the winter and spring and approached by 'Grasslands Nui' and 'Kleppe' from SD3 (mid-winter) (Figure 5.23). This is the same number identified in Chapter 4, Section 4.3.8.1, (Figure 4.18). Thus, 4HS is the shortest possible duration from FI until final leaf appearance. The LN_{postFI} was greatest when FI occurred under short Pp, calculated at ~6.5 leaves and reduced towards 4 as Pp increased, effectively utilising the same mechanism as wheat (Brooking *et al.* 1995; Jamieson *et al.* 1995a). Assuming that ~2 leaves are produced post TS, the change in final leaf number is a result of changes in the number of leaves that emerge between FI^{HS} and TS^{HS}.

5.1.12 Modelling duration from FI to FL emergence

5.1.12.1 Modelling FLN

A shortfall of the FLN approach in *Sirius*, is that the potential number of leaves left to emerge is set by the number of primordium at V_{sat}, following which the leaves to emerge potentially decreases if V_{sat} occurs in long Pp (Jamieson *et al.* 1998b; He *et al.* 2012). However, when perennial ryegrass reaches V_{sat}, the number of potential leaves to emerge remains infinite as primordium continue to accumulate at the vegetative rate, until the genotype specific Pp_{base} is achieved. The mechanism that determines FLN in perennial ryegrass is the Pp response that adjusts the HS duration between FI and TS. This effectively determines the number of leaves that remain to emerge, as at TS two leaves remain. Thus, FLN becomes the LN_{postFI} added to the leaf number at FI. When the LN_{postFI} is multiplied by the phyllochron, it becomes a Tt target for FL emergence that decreases as Pp at FI increases (Figure 5.28)

The change in the LN_{postFI} was driven by the Pp between FI and TS (Figure 5.23) and calculating LN_{postFI} requires a genotype specific response to Pp (Figure 5.28). The genotypes showed two different responses where:

1. 'Medea', 'Grasslands Nui' and 'Grasslands Impact' achieved FI at a similar time, but showed different Pp responses (regression slope) to ultimately produce a different LN_{postFI}, which produced different final leaf emergence dates and,
2. 'Kleppe' that showed a larger Pp_{base} for FI, followed by a rapid decline in the additional LN_{postFI} as Pp increased.

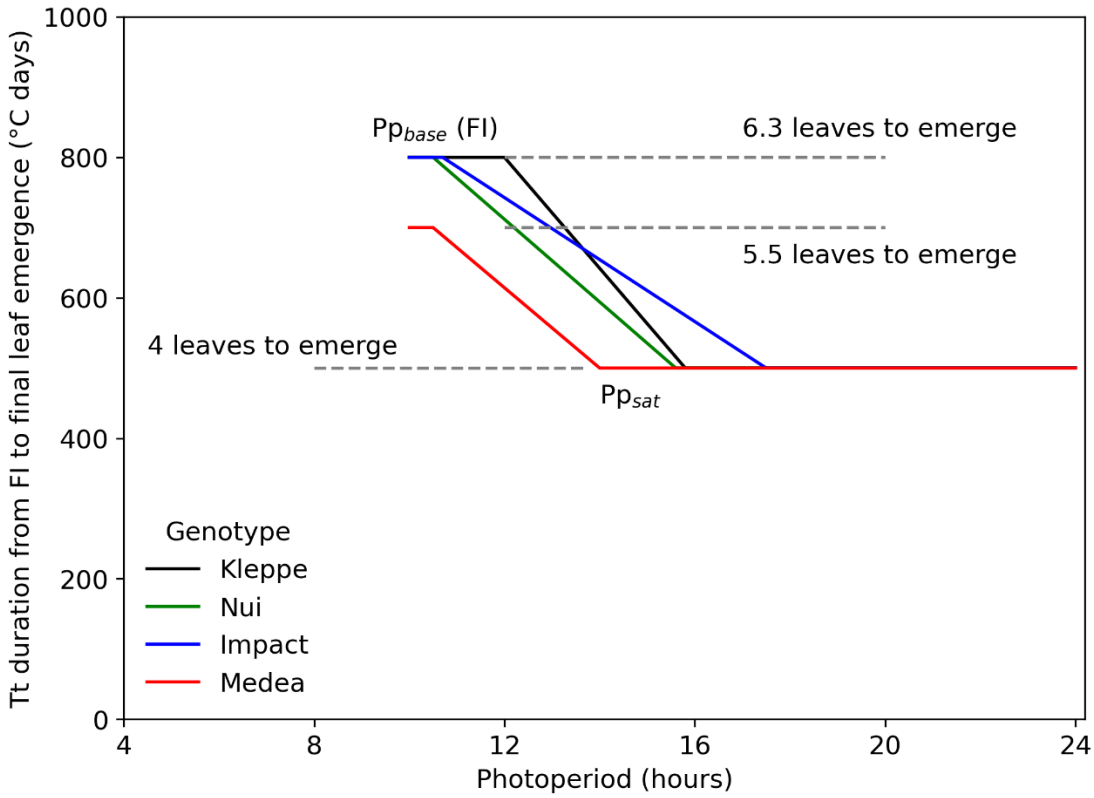


Figure 5.28. Thermal time (Tt) requirements of four perennial ryegrass genotypes relative to the number of leaves to emerge following floral initiation (FI) as Pp increases during spring, data based on a single experiment with five sowing date at Lincoln, New Zealand in the 2019/20 growing season.

Many authors state that differences in Pp required for FI determine much of the variation in heading date (Cooper 1952, 1956b; Heide 1994; Aamlid *et al.* 2000; Hurley *et al.* 2006). This was correct when comparing 'Kleppe', with the remaining genotypes, however it was incorrect for separating 'Medea', 'Grasslands Nui' and 'Grasslands Impact' where FI occurred at the same Pp and the Pp response between FI^{HS} and TS^{HS} determined the timing of final leaf emergence.

Utilising this approach contains a self-correcting mechanism for errors in the prediction of FI. For example, if the date of FI is overestimated, additional time is spent vegetative and FI occurs in longer Pp, which in tern, reduces the number of leaves that will emerge post FI, thereby reducing the duration to FL and vice versa.

5.1.12.2 Modelling via modified Tt.

The Pp function in ARCWHEAT1 requires modifications to Equation 6. Base Pp (Pp_{base}) prior to DR is genotype specific so no progression towards flowering occurs until Pp_{base} is achieved in lengthening Pp, even if the vernalisation function is saturated. Additionally, the Pp_{opt} must be reduced from 20 h to a genotype specific parameter ranging from ~13.5-16 h. Additionally, genotypes require specific Tt targets for the FI-FL duration ranging from 325 – 620°C days.

Modelling summary

Both FLN and modified Tt achieved prediction of FL emergence within a range of ±6 days. Given that perennial ryegrass genotypes are a collection of outcrossing plants where final leaf emergence and flowering can span ~20 days (Chynoweth 2012; Abel & Boelt 2018), this level of error appears acceptable.

5.1.13 Duration from final leaf to flowering

For SD1-3, the duration from final leaf until flowering (anthesis) (FL-AN) was conservative within genotypes 'Medea', 'Kleppe' and 'Grasslands Nui' (Table 5.16). However, the duration was shorter for 'Kleppe' suggesting a genotype specific duration for accurate estimation. Hurley *et al.* (2006) suggested that later season genotypes had shorter Tt duration from DR to ear emergence compared with early season genotypes. However, they did not count leaf number to assess possible Pp influences on the duration. The conservative nature from FL-AN within a genotype is consistent with wheat where three phyllochron quantifies the duration from FL-AN (Jamieson *et al.* 1998b; Jamieson *et al.* 2007). For this experiment 3.5 phyllochron was appropriate for 'Kleppe' while all other genotypes required 4.5 phyllochron, which suggests the duration is longer in perennial ryegrass than wheat.

For 'Grasslands Impact' (SD1-3) and 'Medea' in SD4-5, the duration from FL-AN reduced as sowing was delayed (Table 5.16). First, changes in T_b were investigated, but the differences between treatments remained as T_b was increased from 0–10°C thus, there appeared no reason to shift away from T_b = 0°C. It could be expected that a higher T_b may reduce the variability between treatments as generally the night temperatures did not

fall below ~7°C for the FL-AN timeframe (Bonhomme 2000). However, 'Grasslands Impact', SD1-3, experienced similar temperature cycles to 'Grasslands Nui' and 'Kleppe' where the FL-AN duration was stable. The use of a T_b=0°C for the FL-AN duration is supported by other Gramineae, such as oats (Sonego 2000) and wheat (Jamieson *et al.* 1998b). Secondly, potential dry matter production was investigated to assess a growth mechanism across sowing dates (Table 5.17). Changes in DM production did not match changes in FL-AN duration, thus given genotype differences occurred at similar timeframes, the process of DM production is unlikely. However, the reduction in FL-AN duration was associated with shorter stems that produced fewer spikelets per stem (Table 10.1), both of which were related to a reduced duration from FI^{HS}-TS^{HS} (Table 10.1, Appendix 16). A reduction in the number of spikelets was also shown by Rawson (1970) as the FI-TS duration reduced for both triticale and wheat. Rawson (1971) demonstrated that more spikelets were formed in controlled environment conditions during an 8 h Pp, compared with 16 h Pp, and that the duration from FI-TS increased in shorter Pp for both species. Since the duration from FI^{HS} to TS^{HS} was influenced by Pp, the reduced number of spiklelets was probably driven by a Pp response and indicates a genotype response, potentially useful for seed yield estimation.

Concurrently, for 'Grasslands Impact' and 'Medea' in SD4-5, reproductive stem length followed a similar reducing pattern to spikelet number, possibly related to the duration from FI^{HS} to TS^{HS}. This potentially provides a mechanism where a shorter seed head and final internode emerge in a reduced duration to advance flowering date during longer Pp.

Slafer *et al.* (1995) suggested photoperiod during early FI could influence the culm length of wheat, primarily through leaf number and internode length, but did not provide a mechanism for changes in internode length. Gibberellic acid (Gibberellin, GA) has long been known to promote stem elongation of grasses through increasing cell division and expansion, thereby determining potential plant height (Cutter 1971; Kende *et al.* 1998). During the 'Green Revolution' it was semi-dwarf wheat and rice with impaired GA biosynthesis that allowed for increased grain yields, primarily through increased harvest index resulting from shorter stems (Austin *et al.* 1989; Peng *et al.* 1999; Hedden 2003). Recently, Nagai *et al.* (2020) proposed a series of genes that either enhance stem

extension (*ACCELERATOR OF INTERNODE ELONGATION (ACE1)*) or antagonise stem extension (*DECELERATOR OF INTERNODE ELONGATION (DEC1)*) in rice that influence overall stem length through changes in the intercalary meristem due to GA expression. The authors show these genes are conserved and function in other grasses, including *Brachypodium* and barley, where plant height and internode length were reduced when *DEC1* was upregulated. It seems likely that these genes may be present in other Gramineae, such as perennial ryegrass. This provides a mechanism for shorter plants in response to environmental conditions.

In perennial ryegrass, long Pp has been shown to increase the production of GA_5 and GA_6 (King & Evans 2003; MacMillan *et al.* 2005) and sometime later GA_1 and GA_4 can be found at the stem apex, such that they may induce the transition from vegetative to reproductive development. Concurrently, GA_1 and GA_3 concentrations increase in the tissue below the stem apex. Many of the different GAs have different tasks and it is possible that the concentrations of the various GA compounds, and/or there inactivation in response to Pp, may have resulted in shorter stems (Rademacher 2000). Since GA production or antagonism can be regulated by Pp, there is potential that reduced duration from final leaf to flowering is genotype driven in response to Pp, possibly in association with other phytohormones.

5.1.14 Modelling duration from final leaf to flowering

For both modelling methods, the genotype specific durations from FL-AN for 'Grasslands Nui' (560°C days) and 'Kleppe' (415°C days) the can be added as a Tt target beyond the duration to FL (Table 5.16). 'Medea' requires a split approach, where sowing within the 'normal' autumn sowing dates can be treated the same as 'Grasslands Nui' (600°C days). However, for 'Grasslands Impact' and spring sowings of 'Medea', the duration shortens relative to the duration between FI^{HS} and TS^{HS}. Thus, a Pp calculation utilising the mean Pp between FI^{HS} and TS^{HS} can be implemented to reduce the Tt target from ~600°C days (Equation 29). Thus, 'Grasslands Impact' starts out treated as 'Grasslands Nui' but finishes as 'Kleppe' when exposed to common Pps between FI-TS.

5.2 Conclusions

This chapter quantified the Pp response of four perennial ryegrass genotypes. A genotype specific Pp determined the timing of FI while the Pp between FI and TS determined the duration from FI until final leaf emergence. Some individual conclusions are:

- For 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact' flowering required vernalisation, for which the response was saturated only when planting occurred prior to mid winter. Medea, acted as a facultative long day plant and flowered from all sowing times.
- The number of main stem leaves produced by all genotypes decreased as sowing was delayed from March until June.
- Floral initiation was achieved at a genotype specific, lengthening Pp, ranging from 10.3-12 h.
- The number of leaves produced post FI determines the duration from FI until final leaf emergence. The response reduced from ~6.5 leaves towards 4 as the Pp between FI and TS increased towards a genotype specific saturating Pp.
- Genotypes demonstrated a range of Pp responses where 'Medea' and 'Kleppe' showed Pp_{sat} soon after Pp_{base} (~2 h) compared with 'Grasslands Impact' where Pp_{sat} was calculated as ~6.5 h after Pp_{base}.
- Final leaf date could be accurately estimated from an estimate of FLN multiplied by the phyllochron where;
    - FLN could be estimated as TS^{HS} plus 2,
    - TS^{HS} could be estimated from FI^{HS} plus 2-4.5HS depending on genotype specific Pp response
    - FI^{HS} could be estimated as the HS at which both vernalisation requirements have been met and increasing photoperiod exceeds a genotype specific target.
- For spring sown 'Medea', the minimum number of main stem leaves was 9 and when multiplied by the phyllochron provided accurate estimation of final leaf emergence date.
- Flowering date can be estimated as a genotype specific Tt target after final leaf appearance.

6 GENERAL DISCUSSION AND CONCLUSIONS

6.1 Introduction

The aim of this thesis was to create a predictive model of the time to flowering for perennial ryegrass. To do this required the phenological development of perennial ryegrass to be quantified in relation to different temperatures and photoperiods. This was achieved through a series of controlled environment and field experiments. Firstly, four controlled environment experiments separated genotypes into groups based on temperature and Pp responses, from which four genotypes with contrasting induction requirements were selected for further analysis. Secondly, genotypes were separated based on temperature and Pp responses during primary induction via eight temperature and Pp experiments located in controlled environment chambers. Finally, the secondary induction response and the durations between FI-TS, TS-FL and FL-AN were quantified. This was achieved utilising a field experiment where a combination of sowing date and genotype responses altered the timing of FI, allowing a mechanistic model to be produced.

A part of the aim was to use the modelling approaches of ARCWHEAT1 and *Sirius* as a framework to understand and quantify environmental responses of perennial ryegrass. This involved quantifying the genetic differences that arise among perennial ryegrass genotypes from different centres of origin. This chapter summarises the key phenological results from individual chapters and relates these to genetic differences between genotypes. It links these with common vernalisation genes found in winter wheat. The implication being that similar activity occurs in perennial ryegrass, potentially via homologs of the same gene or by an unknown series of genes that express similar activity.

Briefly the three main genes are referred to as, *VRN1*, *VRN2* and *VRN3*. The expected pattern of expression from wheat is: *VRN3* promotes flowering and must be expressed at sufficient levels to allow FI. *VRN3* is blocked by *VRN2* expression. *VRN1* is upregulated by exposure to cool temperatures where a critical target blocks *VRN2*. *VRN2* is downregulated in short Pp reducing the amount of *VRN1* required to achieve the *VRN1*

expression target required to upregulate *VRN3* (Yan *et al.* 2006; Brown *et al.* 2013; Herridge *et al.* 2021).

6.1.1 Flowering in perennial ryegrass

Chapter 1 sourced germplasm from a range of latitudes and included four experiments to quantify the mechanisms of flowering in perennial ryegrass.

Firstly, and unexpectedly, many accessions achieved flowering without vernalisation in Experiment 1 (constant 18°C, 20 h Pp). Many flowering genotypes were from low latitudes and achieved at least 50% of plants flowering, suggesting a Pp response where intensity of flowering decreased as Pp reduced in Experiments 2 (constant 18°C, 14 h Pp) and 3 (constant 18°C, 11 h Pp). This demonstrated potential for Pp, perhaps via direct Pp influence on *VRN3*, to override the vernalisation pathway and upregulate *VRN3* to allow flowering to proceed (Yan *et al.* 2006). Alternatively, this response could be explained by a facultative vernalisation response where vernalisation progressed slowly and flowering eventually occurred. However, 'Grasslands Nui' did not flower in constant 18°C, 17 h Pp during the vernalisation experiments, even after 11 months exposure suggesting the role of *VRN3* is more likely.

Secondly, Experiment 4 (4°C for 12 weeks followed by 18°C, 20 h Pp) showed that many mid and high latitude accessions were obligate for vernalisation to flower in 20 h Pp, while some high latitude lines did not flower in any experiments, e.g. 'Kleppe'. Experiment 4 showed there were differences in either duration of exposure or the absolute low temperature requirements to upregulate *VRN1* and thus suppress *VRN2* expression during 20 h Pp. Concurrently, low latitude lines flowered in Experiments 1, 2 and 4 where flowering proceeded in response to Pp, e.g. 'Medea', but for many, changes in the final number of main stem leaves suggested a vernalisation response was required to predict time of flowering. Thus, within the germplasm three main mechanisms that control flowering where identified;

(i) those where 20 h Pp allowed progression to flowering (e.g. 'Grasslands Nui'), possibly through *VRN3* overcoming the *VRN2* block on flowering, but required vernalisation, *VRN1* expression, at shorter Pp,

(ii) those that required vernalisation, *VRN1* expression, and showed a strong *VRN2* suppression of flowering in long Pp (e.g. 'Kleppe'),

(iii) those that likely flower in response to Pp alone with limited or no *VRN2* expression (e.g. 'Medea' and Kangaroo Valley).

The data suggested differences in at least three genes that interact to induce flowering, which is consistent with winter and spring wheat.

6.1.1.1 Implications

Unexpectedly several accessions flowered in long Pp without exposure to cold temperatures. This has implications for plant breeders looking to manipulate seed lines in controlled conditions under long Pp which hastens development and allows multiple generations per year. For genotypes such as 'Medea', this approach appears feasible as Pp determined FI, but for genotypes that usually require vernalisation when grown in field conditions e.g. 'Grasslands Nui', propagation in long Pp may over time result in the selection of different mechanisms for flowering. Thereby, reducing the end user advantages of vernalisation. For seed producers, this mechanism raises potential to produce seed outside the standard planting window utilising long Pp or potentially manipulating the Pp response. For example, GA application has been shown to replace the requirement for long Pp in vernalised plants (King & Evans 2003; MacMillan *et al.* 2005). Thus, GA could potentially be applied to non-vernalised genotypes that show the long Pp induction trait and allow seed production from spring sowings where natural Pp does not induce FI.

6.1.2 The phenophases/genetic responses

Experiments 5-13 were conducted across a range of both controlled environment chambers and field conditions. As expected, crops sown in decreasing photoperiod in the field took longer to reach flowering compared with those sown during winter. Autumn sown crops produced more leaves prior to the onset of FI than later sown crops as a mechanism to ensure flowering in the summer months. From autumn sowings, 'Medea' followed the same pattern and flowered in summer but, also flowered from spring

sowings, indicating a facultative response was probably initiated by direction of Pp change.

The duration of phenophase 1, germination to emergence, was dependent on soil temperature alone and conformed to a single Tt target when near seed temperature was used (T_b=0°C). The Tt target was smaller when emergence occurred in potting mix than in the field, presumably based on differences in soil moisture availability or the bulk density differences of the soil media. Tt based on air temperature was less accurate and overestimated Tt accumulation in winter, indicating that seeds respond to the environment around them and soil temperature provided the most accurate prediction of germination and emergence.

Following crop emergence, seedlings respond to both temperature and Pp although the shoot apex remains under the soil surface until floral initiation and the beginning of stem extension. Experiments 5-13 allowed the genetic variation in response to cool temperatures (*VRN1*) and Pp during exposure (*VRN2*) to be separated.

'Medea' was obligate for long, or lengthening, Pp where plants held in constant 8 or 11 h Pp did not achieve FI. 'Medea' does not require vernalisation for flowering when Pp exceeds 14 hours. For 'Medea', V_{sat} was achieved faster as temperature increased to 18°C and was achieved in all treatments at the end of the juvenile phase, HS4. Therefore, modelling of the vernalisation response utilised a linear relationship between 4 and 18°C in both short and long Pp. However, FI could be modelled from the end of the juvenile phase based on the current HS and Pp response only e.g. in long Pp the FLN = FI^{HS}+4. The juvenile phase ended at ~HS4, regardless of temperature or Pp, and suggests a constant accumulation of *VRN1* expression/HS, becoming primary induced at ~HS4.

Thus, it is hypothesized that 'Medea' either;

I. does not contain an effective *VRN2* type gene, or
II. *VRN1* expression occurs at a constant rate to achieve V_{sat} at HS4, or
III. *VRN3* was upregulated by a Pp of 14 h or longer.

Additionally, Pp genes, detected the change in Pp direction after the shortest day, determining the time of FI in short Pps. In Experiment 13, 'Medea' achieved FI shortly after the shortest day when Pp was 10.5 h, whereas no flowering was recorded at constant 18°C, 11 h Pp in Experiment 3. FI was triggered following HS4 and when the Pp was lengthening above 10.5 h. These results indicate that genotypes with similar genetics to 'Medea' can flower from spring and summer sowing when Pp is lengthening. However, when Pp was decreasing (e.g. autumn sowing in Experiment 13), flowering did not occur until the following spring, which shows an obligate lengthening/long day response.

However, genetic variation was shown in Experiment 13 where ~70% of 'Medea' plants flowered from the late spring sowing, suggesting some plants have either *VRN2* activity in long Pp or temperatures were not cold enough to induce a *VRN1* response to suppress the *VRN2* in these plants.

In 'Kleppe', flowering followed the expected pattern demonstrating a clear vernalisation response followed by a photoperiod requirement. Short Pp (8 h) down regulated *VRN2*, and V_{sat} was achieved after exposure to 6 weeks of temperatures less than 12°C. Subsequently, FI occurred when a Pp of ~12 h or longer was encountered and when seedlings had produced at least 5 leaves. However, in long Pp, flowering was repressed (possibly by *VRN2* expression) such that temperatures of 4°C, for at least 10 weeks, were required which may then be sufficient for *VRN1* to be upregulated sufficiently to suppress the activity of *VRN2*, allowing *VRN3* to be upregulated in response to long Pp. This is consistent with the proposed winter wheat response. For modelling in short Pp, the duration required to reduce the number of leaves post FI (V_{sat}) and therefore temperature effectiveness, was the same for temperatures between 4-12°C. Therefore, there was a plateau relationship with cardinal temperatures of -4, 4, 12 and 13°C appearing appropriate. However, as Pp lengthens, the response requires an upper temperature limit with a increased *VRN1* expression to downregulate the increasing *VRN2* expression. In the absence of further data, the relationship to predict the maximum temperatures for vernalisation, per hour of Pp, was assumed to be linear between 12°C in 8 h Pp and 4°C in 17 h Pp, while concurrently the duration required increased from 6–10 weeks. In no experiment did 'Kleppe' demonstrate an ability for *VRN3* to be upregulated by Pp alone.

Thus, 'Kleppe' appears to represent germplasm from Northern Europe and confirms the expected patterns described previously (Cooper 1960; Evans 1960; Heide 1994; Aamlid *et al.* 2000)

'Grasslands Nui' showed a similar response to 'Kleppe' with a clear vernalisation response, followed by the requirement of long photoperiod in most experiments. However, in Experiment 1, constant 18°C, 20 h Pp, 'Grasslands Nui' achieved FI in the absence of cool temperatures which suggests differences from 'Kleppe' in the *Pp*/*VRN3* pathway. For 'Grasslands Nui' in short days, temperatures of ≤12°C for a minimum of 6 weeks saturated the vernalisation response, and potentially up regulated *VRN1*. In long days, 8°C was adequate to up regulate *VRN1* and suppress *VRN2* after 8 weeks of exposure and allow FI to proceed when Pp was above 10.5 h. Thus, compared with 'Kleppe', there are differences in the activity of *VRN1* (more active) and *VRN2* (less active) that allow Grasslands Nui' to become primary induced at warmer temperatures during long Pps, e.g. the autumn and spring periods. Thus, 'Grasslands Nui' showed a vernalisation response alike 'Kleppe' while the Pp response was comparable with 'Medea'.

For 'Grasslands Impact' the same general genetic response was shown as 'Kleppe' and 'Grasslands Nui' where short days allowed *VRN1* upregulation at temperatures up to 12°C. However, V_{sat}, was achieved faster in warmer temperatures. Therefore, modelling of the vernalisation response utilised a linear relationship between 4 and 12°C. In 17 h Pp, 12°C was eventually cold enough to upregulate *VRN1* and suppress *VRN2* to allow flowering, but 12 weeks duration was required to achieve 50% flowering. 'Grasslands Impact' demonstrated an obligate requirement for vernalisation followed by relatively short Pp to induce FI. However, FL emergence was delayed due the production of more leaves between FI and TS, indicating that the Pp response post FI was responsible for delaying FL emergence.

The vernalisation experiment demonstrated that vernalisation operates as a switch that provides competence for flowering sometime in the future. This follows the principles of primary and secondary induction followed by Heide (1994). In all experiments where V_{sat} occurred in short or decreasing Pp, FI occurred only after exposure to a genotype specific

'long' Pp. This supports the idea that the *VRN1* gene alone cannot upregulate *VRN3* and induce FI. A result supported by Herridge *et al.* (2021) who showed similar *VRN1* expression levels during vernalisation in both early and late flowering genotypes where greater expression levels in early genotypes could be expected if *VRN1* directly upregulated *VRN3* to produce an earlier flowering response.

Thus, it appears the functionality of the genes present in perennial ryegrass are in common with those from wheat. However, unlike winter cereals, 'Kleppe', 'Grasslands Nui' and 'Grasslands Impact' are obligate for both vernalisation and long Pp. Therefore, unlike wheat, *VRN1* expression requires cold conditions and does not accumulate at a base rate as suggested for wheat by Brown *et al.* (2013). Thus, *VRN1* expression is increased by cold temperature and represses *VRN2* where plants can enter a 'holding' phase until appropriate Pp up regulates *VRN3* to induce FI.

While the pattern of gene expression appears similar to that of wheat, and could potentially be modelled as such, it remains possible that *VRN3* is regulated by other as yet unknown genes to allow flowering in long Pp without *VRN1* expression. For example, the GIGANTEA(GI) genes were initially identified as having a role in the Pp pathway as a component of the circadian clock and may directly act on COSTAINS genes with potential to influence *VRN2* and subsequently *VRN3*. Additionally, *VRN3* is likely expressed in leaves where it travels to the stem apex where potential repression could occur via other genes e.g. *LpFLT1* (Arojju *et al.* 2016) until Pp_{base} is achieved.

6.1.2.1 Potential implications

Differences in *VRN2* responses lead to different vernalisation responses between genotypes as the Pp lengthens. Of interest for plant breeders is the duration below threshold temperatures required to up regulate *VRN1* in long Pp. For example, cool night time temperatures are often observed in spring when Pps are lengthening, allowing the accumulation of *VRN1*. Concurrently as the Pp increases *VRN2* expression increases and colder temperatures of increased duration are required to reach the *VRN1* threshold to suppress *VRN2*. Potentially an increased expression of *VRN2* could be utilised to suppress flowering if *VRN2* expression could be upregulated in Pps of ~10 h or less.

In the South Island of New Zealand, most seed crop plantings are vernalised by upregulation of *VRN1* during late autumn and winter when *VRN2* expression is suppressed by short Pp, and many genotypes are fully vernalised before the shortest day. Therefore, the Pp response that upregulates *VRN3* determines the flowering time of perennial ryegrass. For spring planting, PI does not occur as *VRN2* expression increases as the Pp lengthens, effectively increasing the target level of *VRN1* required to overcome *VRN2*. Changes in the alleles around *VRN2* and *VRN3* determine the flowering intensity in marginal conditions e.g. 'Grasslands Impact' achieved low level flowering in SD4 compared with 'Kleppe' which did not flower in SD4.

For Northern areas of New Zealand, *VRN1* upregulation occurs over an extended timeframe such that warm winters delay the onset of FI due to *VRN1* restrictions. However, modelled FL emergence dates were similar to the South Island due to warmer temperatures accumulating the target Tt required for leaf appearance in a shorter duration than at South Island locations.

Experiment 13 investigated the Pp response from five sowing dates. When flowering occurred, the Pp response determined the duration from FI to flowering via two mechanisms;

I. the timing of the critical Pp at which FI occurred (Pp_{base}), and
II. the slope of the Pp response which determines the number of leaves produced post FI (LN_{postFI}).

Much of the past literature has focused on the critical Pp required for FI, however both responses are critical to predict the developmental stages post FI. For example, in SD1 'Medea', 'Grasslands Nui' and 'Grasslands Impact' all achieved FI at approximately the same Pp. However, 'Grasslands Impact' produced more leaves between FI and TS which increased the duration to FL emergence. The use of linear equations allowed for the separation of genotypes and the prediction of the LN_{postFI}, which becomes a Tt target then multiplied by the phyllochron.

Since *VRN3* is expressed in long days after vernalisation (Herridge *et al.* 2021), it is hypothesised that *VRN3* is the likely gene determining the time of FI and TS formation. It is possible that a promoter gene is present given that a critical Pp is required. Thus, following upregulation of *VRN1*, and the obtainment of Pp_{base}, *VRN3* expression determines the timing of FI. Since the duration from FI^{HS} to TS^{HS} was shortest in long Pp, and reduced the LN_{postFI} accordingly, the upregulation of *VRN3* is quantitative and occurs in response to Pp. This is supported by Skøt *et al.* (2011) who showed allelic variation of *VRN3* was associated with differences in flowering time across a range of European perennial ryegrass genotypes where the response will reduce the HS between FI and TS. Therefore, expression of *VRN3* could be modelled to predict TS as per Brown et al. (2013).

No relationship was shown between location of origin and the Pp response, thus the Pp response must be derived from plants grown in the field.

The duration from FL-AN followed a genotype specific duration for 'Grasslands Nui' (560°C days) and 'Kleppe' (415°C days) that can be treated as a Tt target beyond FL (Table 5.16). 'Medea' requires a split approach, where 'normal' autumn sowings can be treated the same as 'Grasslands Nui' (600°C days). However, for 'Grasslands Impact' and spring sowings of 'Medea', the duration shortens relative to the duration between FI^{HS} and TS^{HS}. Thus, a Pp calculation utilising the mean Pp between FI^{HS} and TS^{HS} can be implemented to reduce the Tt target from ~600°C days (Equation 29). It is likely this response is genetically controlled by photoperiod and a consequence of changes incurred during the FI-TS stages. This potentially provides a mechanism where a shorter seed head and final internode emerge in a reduced duration to advance flowering date when FI-TS occurs in a longer (closer to summer) Pp, potentially acting as a drought escape mechanism for genotypes from lower latitudes of origin.

6.1.3 Modelling key phenophases

Three of the four genotypes tested were obligate for vernalisation to achieve flowering, while 'Medea' appeared to be primary induced at HS4 regardless of temperature. The genotypes showed responses common to both ARCWHEAT1 and *Sirius,* and utilising components of both modelling techniques enabled a modified approach to predict V_{sat}

and final leaf emergence of the different genotypes. However, unlike the ARCWHEAT1 and *Sirius* approaches, V_{sat} or primary induction, does not lead to changes on the apical meristem and primordium production continued at the vegetative rate until a genotype specific Pp_{base} was achieved. Thus, both techniques required the addition of a genotype specific Pp_{base} to predict the onset of FI, which occurs sometime after genotypes are primary induced.

The ARCWHEAT1 approach predicted FL and flowering date while demonstrating genotype differences in response to temperature and photoperiod, but does not explain the physiological processes occurring within the plant. Utilising a modified mechanistic approach, based on the number of leaves to emerge between key developmental phases, provided a detailed explanation utilising the co-ordinated events occurring on the stem apex. Initially leaf primordium are produced at the same time as leaves emerge. There were approximately two primordium present on the stem apex at emergence. Primordium production followed the same pattern as wheat, ($P \approx 2HS+2$) until HS8 when the faster plastochron lead to an accumulation of 10-12 primordium. Following HS8 the plastochron reduced to 1/HS until either FI was obtained or the experiments finished. Presumably the steady state relationship would continue for the lifespan of each tiller. This relationship was constant across the four genotypes investigated in both controlled environment and field experiments. It allows for the calculation of primordium number when HS is known. Following FI the plastochron increased as spikelet differentiation occurred at a genotype specific 'long' Pp. Following FI, the number of leaves to emerge followed a relationship similar to the Pp response in *Sirius*, where a saturating Pp was utilised to reduce the number of leaves to emerge towards a minimum of four. Effectively this reduced the FI-TS duration as Pp increased. The Pp response accurately characterised differences between genotypes allowing estimation of FL to within six days, even when FI occurred at similar Pp for three genotypes but FL emergence occurred across a range of 20 days.

In summary, modelling of development in perennial ryegrass requires functions to compute progress based on vernalisation and Pp of which zero progress towards flowering is made until base levels of both vernalisation and Pp are achieved (Table 6.1).

Table 6.1 Stage and phase names along with the key requirements for model progression though the phenophases from seed germination until maturity.

Stage Name	Phase Name		Phase duration:
Germination	**Phase 1.**	Emergence	188 ± 10°C days
Emergence	**Phase 2.**	Primary induction	Genotype specific vernalisation target where cooler temperatures are required as Pp increases
Vernalisation complete	**Phase 3.**	Secondary induction	Achieved at genotype specific lengthening photoperiod
Floral initiation	**Phase 4.**	Ear development	Decreases at a genotype specific rate based on photoperiod, generally 2 - 4.5 ± 0.36HS
Terminal spikelet	**Phase 5.**	Stem extension	2 - 2.5 ± 0.28 HS
Final leaf appearance	**Phase 6.**	Ear emergence	Genotype specific duration, generally 400 – 600 ± 29°C decreasing in certain genotypes
Flowering	**Phase 7.**	Seed filling	517 ± 13°C days (Chynoweth & Moot 2017)
End Grain Fill			

6.1.3.1 Model progression can be summarised by:

1. Tt with a T_b of 0°C explained the duration from sowing to emergence.
2. Genotypes require a genotype specific vernalisation target below 12°C in 8 h Pp decreasing to a genotype specific maximum temperature as Pp increases where vernalisation acts as a switch to release the crop to flower
3. FI^{HS} is predicted from current Pp once the vernalisation model reaches V_{base},
4. TS^{HS} is predicted from FI^{HS} and current Pp,
5. FLN is predicted from TS^{HS} where 2-2.5 leaves remain to emerge at TS.
6. Tt, with a T_b of 0°C, explained the duration from FL to flowering where genotype specific Tt target is required. For some genotypes a Pp response is required.

6.2 Implications of these results

6.2.1.1 Grazers and plant breeders

Understanding the genes present and their individual responses involved in reproductive development gives the potential to make targeted selections. For example, the production of non flowering ryegrass may increase spring and summer forage quality through the production of fewer stems. For perennial ryegrass, a non-flowering response requires *VRN3* to remain down regulated. This goal could be achieved via the investigation of alleles on the main vernalisation genes where;

I. continuous *VRN2* expression suppresses *VRN3*, (*VRN2* is present in long Pp),
II. where temperatures are not cool enough to reach a *VRN1* target, and suppress *VRN2* expression, or,
III. where the Pp is short and does not up regulate *VRN3*.

Since all genotypes were vernalised at temperatures of 12°C or less when exposed to short days, it appears that the base vernalisation requirements can be achieved for all genotypes at all locations throughout NZ. Thus, breeding for extreme changes in *VRN1* expression alone may be ineffective. However, investigating variation within *VRN2* expression may provide opportunities to produce non flowering genotypes for northern New Zealand. For example, genotypes 'Medea' (none) and 'Kleppe' (strong) already

express contrasting *VRN2* activity with intermediate results shown by 'Grasslands Impact'. Thus, investigations quantifying the Pp required to down regulate *VRN2* may provide an insight to reduce flowering intensity. Therefore, currently at locations where *VRN1* expression occurs in short Pp, the temperature threshold for the accumulation of *VRN1* expression is high e.g. 12°C in 8 h Pp (Figure 6.1). If variation in the *VRN2* response could be found, such that a doubling in the *VRN1* target expression was required, to suppress *VRN2* expression, then there is the potential to interrupt flowering. For example, in 'Kleppe' in 12 h Pp, temperatures of less than 8°C may be required to induce a *VRN1* response (Figure 6.1). However, this responds requires confirmation via further data points.

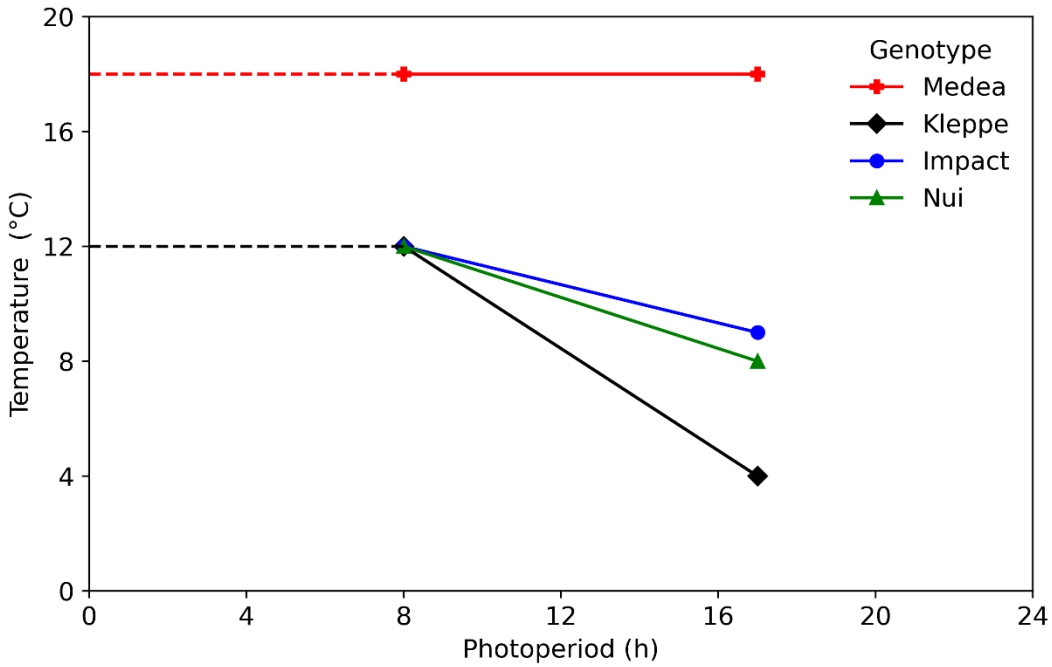


Figure 6.1. Maximum temperature required to induce a vernalisation response at various photoperiods in four genotypes of perennial ryegrass, relationship assumed linear, dashed line assumed extrapolation to zero.

Alternatively, in the south of New Zealand restricted flowering appears difficult to achieve as, days are naturally short in winter, thereby reducing *VRN2* expression while temperatures are naturally cool, thus upregulating *VRN1* expression and summer days are long, enhancing *VRN3* expression. Thus, all criteria for flowering are saturated.

Following spring sowing, a strong *VRN2* response is advantageous as long/increasing Pp reduces the progress towards V_{base} as *VRN2* expression increases until short days are encountered during the following winter. Thus, the first summer has no flowering stems. However, in genotypes such as 'Medea', where *VRN2* appears redundant, spring planting will lead to seed head production in response to long Pp, thus showing a stronger flowering pattern as latitude increases.

Differences were shown between genotypes in the Pp response during the secondary induction phase (*VRN3*). 'Kleppe' showed a strong requirement for a 'critical' Pp of 12 h to induce flowering. If the Pp requirements could be increased, it is possible that flowering intensity could be reduced at northern NZ locations.

6.2.1.2 Seed producers

For optimum seed production, time of flowering should align with high levels of incoming solar radiation and reduce negative climate stresses, such as frost risk during flowering. Since seed yield is associated with the number of seeds/m^2, production of a large number of seed sites per ear is essential for maximising potential seed yield. Spikelet number was maximised when the ear differentiation occurred under short Pp and duration from FI^{HS} to TS^{HS} was longest. This is consistent with previous results from wheat and suggests seed crops should be sown to complete vernalisation and the juvenile phase prior to the shortest day. Therefore, FI would occur at the shortest possible Pp leading to the largest FI^{HS} to TS^{HS} duration. This is particularly important in those genotypes that showed the largest reduction in spikelet number as sowing was delayed ('Medea' and 'Grasslands Nui'). However, these results confirm that many genotypes can be planted into July (in Canterbury, New Zealand) and achieve V_{base} before spring Pp increases lead to *VRN2* upregulation. Additionally, genotypes that show a low *VRN2* expression could be planted later into the spring.

The information compiled in this thesis allows for the estimation of key developmental stages e.g. FI, final leaf appearance and flowering date. For example, FI is estimated based on Pp where the FI^{HS} to TS^{HS} duration influences the number of spikelets per seed head. For optimum spikelet formation, crop nutrition should be non-limiting. Therefore, the

time of the ear development phase can be estimated to ensure producers supply adequate fertility during this phase. In perennial ryegrass seed crops, plant growth regulators (PGR) are commonly applied at Zadok's GS32 (two nodes detectable on the main stem) to reduce the stem length and provide lodging control. Since leaf appearance and nodal elongation are coordinated, GS32 coincides with two leaves remaining to emerge, effectively as the tip of final leaf 2 appears. Therefore, time for PGR application can be calculated as either TS formation or as a Tt target calculated as two phyllochron fewer than FL emergence.

With additional genotype calibration, this modelling framework can provide guidance on in season growth stages utilising real time weather data to guide management decisions based on developmental stage. Additionally, the phenology data could be implemented into a complete growth model to provide a comprehensive growth and development model capable of both biomass and seed yield prediction.

6.3 Knowledge gaps for further research

The analysis during the previous chapters has highlighted the following research gaps:

1. This series of experiments focussed only on newly sown seedlings and did not explore how the flowering mechanisms are reset each summer, as occurs in multi-year crops and pasture systems. Thus, further research to confirm the induction requirement and phenophase timings of multiyear stands is required for modelling pasture development and understanding multiyear seed crops.
2. Further investigation of the vernalisation response in Pps intermediate of 8 and 17 hours would improve the understanding of the how *VRN1* and *VRN2* interact during the primary induction phase.
3. The phenological data produced in this thesis should be incorporated into predictive growth models to build a genotype specific perennial ryegrass seed production model.
4. The results suggest the presence of a gene with the same Pp activity as the wheat *VRN2* gene. Additional research to identify candidates for a *Lolium perenne, VRN2* gene may allow plant breeders to produce non flowering, or reduced flowering intensity, perennial ryegrass genotypes for some environments.
5. This series of experiments has highlighted the role of three vernalisation genes in the flowering process of perennial ryegrass. The production of a database that contains information on genotypes that carry active copies of *VRN1*, *VRN2* and *VRN3*, and any genetic variation within these, would be advantageous to plant breeders and seed producers trying to match genotypes to specific locations.
6. Measuring the expression of *VRN1*, *VRN2* and *VRN3* during primary and secondary induction, through until terminal spikelet formation, would allow confirmation of the hypothesised gene expression pattern and assist plant breeders to produce location specific genotypes.
7. Investigation of how genetic differences of *VRN1*, *VRN2* and *VRN3* influence the flowering response would allow the development of flowering time model based only those genes present.

7 CONCLUSIONS

The research presented in this thesis has confirmed the influences of temperature and Pp on the development of perennial ryegrass using genotypes from different centres of origin. Experiments utilised both controlled environment and field conditions. Some specific conclusions are:

- Most perennial ryegrass genotypes require vernalisation via cool temperatures and/or short Pp to become primary induced. FI occurs only after exposure to long/lengthening Pp that determines when they are secondary induced.
- The duration from sowing to emergence was conservative among genotypes when quantified using Tt calculated from soil temperature with a T_b of 0°C.
- Perennial ryegrass has a juvenile phase of 4-5HS, during which vernalisation can occur but genotypes remain non responsive to long Pp for SI.
- Vernalisation requirements increased with latitude of origin from 0 Vdd in 'Medea' to 56 Vdd in 'Grasslands Impact', to 70 Vdd in 'Kleppe' when grown in 8 h Pp. In 17 h Pp, an increase in exposure to cooler temperatures for longer durations was required.
- FI^{HS} could be estimated as the HS at which both vernalisation requirements were met and by increasing Pp above a genotype specific target, ranging from ~10.5 h for 'Medea', 'Grasslands Nui' and 'Grasslands Impact' up to ~12 h for 'Kleppe'.
- Final leaf emergence date could be estimated from FLN multiplied by the phyllochron where;
    - FLN could be estimated as TS^{HS} plus 2,
    - TS^{HS} could be estimated from FI^{HS} plus 2-4HS depending on genotype specific Pp response,
- The number of leaves produced post FI multiplied by the phyllochron determined the date of final leaf emergence. Flowering date could be estimated as a genotype specific Tt target after final leaf appearance.
- Genotypes demonstrated a range of spring Pp responses affecting the duration from FI^{HS} to TS^{HS}. 'Medea' and 'Grasslands Nui' and 'Grasslands Impact' reached FI

at similar stages but ‘Grasslands Impact’ reached final leaf ~20 days later due to a longer FI-TS duration.

- For spring sown ‘Medea’, the minimum number of main stem leaves was 9 and when multiplied by the phyllochron provided accurate estimation of final leaf emergence date.
- The pattern of vernalisation and Pp responses suggest the same functioning genes are found in perennial ryegrass as wheat where, *VRN3* is upregulated by long Pp and promotes flowering. *VRN3* is blocked by *VRN2* expression, *VRN2* is downregulated in short Pp. *VRN1* is upregulated by exposure to cool temperatures where it blocks *VRN2*.

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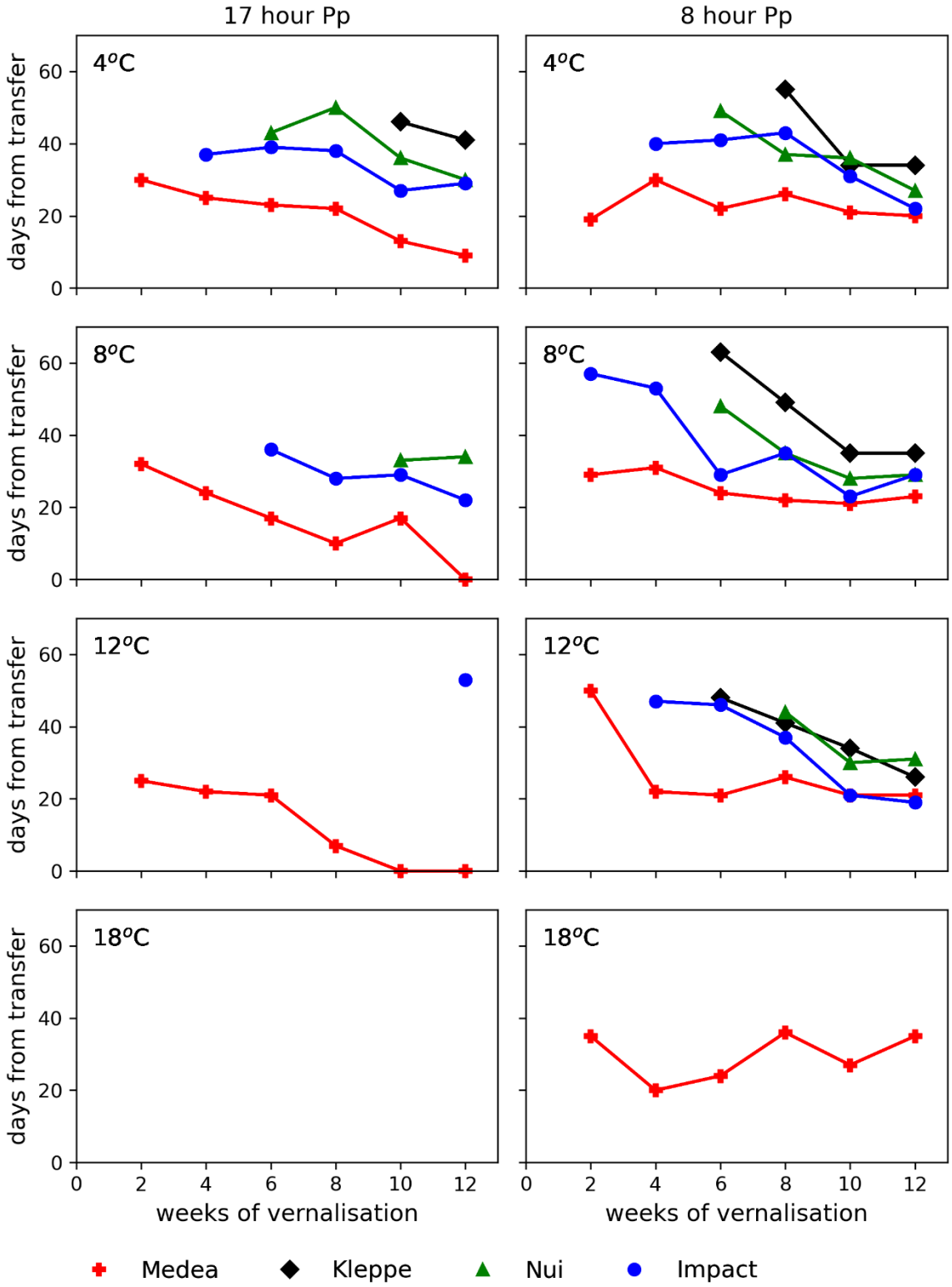
10 APPENDICES

Appendix 1. Percentage of perennial ryegrass plants flowering when grown under four temperature and photoperiod combinations inside controlled environment chambers at Lincoln University, New Zealand.

#	ID/Genotype	Country of origin	Latitude (°)	Percentage plants flowering			
				4 f.b.18°C[1] 20 hr Pp[2]	18°C 20 hr Pp	18°C 14 hr Pp	18°C 11 hr Pp
1	15364	Morocco	33	100	100	-	-
2	A15361	Morocco	34	100	100	**47**	**0**
3	A15323	Algeria	36	93	100	-	-
4	A15371	Tunisia	37	100	93	-	-
5	A14496	Turkey	41	94	81	**7**	**0**
6	A14499	Turkey	41	100	80	**0**	**0**
7	A15769	Portugal	42	88	100		
8	A6012	Spain	43	56	**47**	**25**	**0**
9	A7294	Spain	44	100	**7**	-	-
10	A7283	Italy	46	82	67	-	-
11	A14544	Italy	46	94	75	**0**	**0**
12	A17173	Ukraine	48	63	**0**	**0**	**0**
13	A12434	Netherlands	52	73	**43**	-	-
14	A15338	Denmark	54	94	**44**	-	-
15	A15368	Sweden	58	100	75	-	-
16	A17183	Norway	59	**44**	**25**	**0**	**0**
17	A17184	Norway	60	75	94	**0**	**0**
18	Kangaroo Valley Early		33.5	100	100	100	100
19	Grasslands Nui		37	94	94	**27**	**0**
20	Kleppe		60	**25**	**0**	**0**	**0**
21	Aurora		46	100	75	-	-
22	Medea		36	100	100	79	**0**
23	Grasslands Nui[3]		37	-	-	**7**	**0**
			Mean	85 a	68 b	22 c	-c
			Pvalue	<0.001	<0.001	<0.001	-
			S.E.M	8.54	9.92	7	-

	$LSD_{0.05}$	24.2	28	20.3	-

[1]. 12 weeks at 4°C followed by 18°C, [2]. 6 weeks in darkness followed by 20-hour photoperiod, [3]. Fresh seed collected 3 weeks prior to planting. [3] - not included in individual temperature by Pp treatment. Bold treatments did not reach 50% of plants flowering. Between all chamber comparison, P = <0.001, S.E.M = 4.25, $LSD_{0.05}$ = 12.1.



Appendix 2. Days until final leaf emergence after transfer from four temperature treatment's under 8 or 17 hour photoperiod (Pp) into constant 18°C, 17 hour Pp, of four genotypes of perennial ryegrass when grown in controlled environment chambers at Lincoln University.

Appendix 3. Days after sowing to 50% field emergence of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	10.5	9.8	9.9	10.6	10.2 a
2. 17/04/2019	16.3	16.8	17.8	16.7	16.9 b
3. 14/06/2019	34.4	36.3	35.8	36.1	35.6 d
4. 30/08/2019	22.9	25.1	24.8	22.9	23.9 c
5. 4/10/2019	16.5	15.4	15.4	16.1	15.8 b
Genotype mean	20.1	20.7	20.7	20.5	20.5

Parameter	P value	SEM	$LSD_{0.05}$
Sowing date	<0.001	0.684	2.23
Genotype	0.783	0.473	NS
Sowing date * genotype	0.845	1.143	NS
Means within a sowing date		1.496	3.06

Appendix 4. Curve parameter for logistic regression fitted to field emergence data expressed in days after sowing of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	0.419	0.674	0.751	0.612	0.614 a
2. 17/04/2019	0.415	0.329	0.412	0.379	0.383 b
3. 14/06/2019	0.274	0.311	0.222	0.288	0.274 b
4. 30/08/2019	0.287	0.429	0.338	0.444	0.374 b
5. 4/10/2019	0.262	0.395	0.252	0.273	0.295 b
Genotype mean	0.331	0.428	0.399	0.395	0.388

Parameter	P value	SEM	$LSD_{0.05}$
Sowing date	0.002	0.038	0.125
Genotype	0.498	0.045	NS
Sowing date * genotype	0.815	0.0954	NS
Means within a sowing date		0.101	0.291

Appendix 5. Thermal time, based on 3 cm soil temperature (t_b = 0°C), to 50% (inflection point of logistic regression) emergence of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	185	171	174	185	179
2. 17/04/2019	191	193	200	191	194
3. 14/06/2019	174	186	183	185	182
4. 30/08/2019	190	205	196	185	194
5. 4/10/2019	195	184	186	191	189
Genotype mean	187	188	188	188	188

Parameter	P value	SEM	$LSD_{0.05}$
Sowing date	0.372	5.65	NS
Genotype	0.898	4.42	NS
Sowing date * genotype	0.751	10.3	NS
Means within a sowing date		9.89	NS

Appendix 6. Curve parameter for logistic regression based 3 cm soil temperature (t_b=0°C), used to estimate the duration to 50% emergence of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	0.0254	0.0422	0.0479	0.0382	0.0384
2. 17/04/2019	0.0435	0.0303	0.0374	0.0351	0.0366
3. 14/06/2019	0.0387	0.0432	0.0315	0.0385	0.038
4. 30/08/2019	0.0317	0.0467	0.037	0.0482	0.0409
5. 4/10/2019	0.0225	0.0352	0.0217	0.0236	0.0257
Genotype mean	0.0323	0.0395	0.0351	0.0367	0.0359

Parameter	P value	SEM	$LSD_{0.05}$
Sowing date	0.142	0.00385	NS
Genotype	0.691	0.00430	NS
Sowing date * genotype	0.866	0.00917	NS
Means within a sowing date		0.00962	NS

Appendix 7. Thermal time, based on air temperature (t_b=0°C), to 50% emergence (inflection point of logistic regression) of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	174	164	166	177	170
2. 17/04/2019	186	194	203	191	193
3. 14/06/2019	248	266	263	260	259
4. 30/08/2019	198	219	215	198	207
5. 4/10/2019	186	175	174	182	179
Genotype mean	198	204	204	202	202

Parameter	P value	SEM	$LSD_{0.05}$
Sowing date	<0.001	6.25	20.39
Genotype	0.801	4.59	13.25
Sowing date * genotype	0.785	10.9	31.18
Means within a sowing date		10.3	29.62

Appendix 8. Date of floral initiation of four perennial ryegrass genotypes sown on five dates near Lincoln, New Zealnd in the 2019/2020 growing season. Dates converted to day of year for statisticial analysis.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	25/07/2019	4/09/2019	30/06/2019	17/07/2019	27/07/2019
2. 17/04/2019	21/08/2019	4/09/2019	8/08/2019	6/09/2019	25/08/2019
3. 14/06/2019	10/09/2019	11/10/2019	16/09/2019	3/10/2019	25/09/2019
4. 30/08/2019	5/11/2019	-[1]	-	-	
5. 4/10/2019	13/12/2019	-	-	-	
Mean (SD1-3)	18/08/2019	16/09/2019	8/08/2019	29/08/2019	26/08/2019
Mean (SD1-5)	27/09/2019				

Parameter	P value	SEM	$LSD_{0.05}$
Medea only[2]	<.001	6.39	20.83
Sowing date			
All genotypes – SD1-3 only[3]			
Sowing date	0.003	4.93	19.35
Genotype	<.001	4.06	12.05
Sowing date * genotype r	0.023	7.83	23.38
Means within a sowing date		7.03	20.88

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

Appendix 9. Main stem flowering (%) of four perennial ryegrass genotypes sown on five dates at Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	100	100	100	95.8	98.9
2. 17/04/2019	100	100	100	100	100
3. 14/06/2019	100	95.8	95.8	95.8	96.9
4. 30/08/2019	79.2	0	12.5	41.7	33.3
5. 4/10/2019	64.9	0	8.33	8.33	20.4
Genotype mean	88.8	59.2	63.3	68.3	69.9

Parameter	P value	SEM	$LSD_{0.05}$
Sowing date	<0.001	1.93	6.29
Genotype	<0.001	1.45	4.18
Sowing date * genotype	<0.001	3.40	9.76
Means within a sowing date		3.23	9.34

Appendix 10. Accumulated number of leaves produced on the main stem of reproductive plants of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	15.6	18.6	16.3	17.0	16.9 a
2. 17/04/2019	10.8	14.1	11.4	13.1	12.3 b
3. 14/06/2019	8.46	11.1	9.66	10.5	9.92 c
4. 30/08/2019	9.37	-[1]	*15.0*	*15.6*	
5. 4/10/2019	9.49	-	*14.0*	*15.0*	
Mean (SD1-3)	11.6 a	14.6 d	12.5 b	13.5 c	13.0
Mean (SD1-5)	10.92				

Parameter	P value	SEM	$LSD_{0.05}$
<u>Medea only[2]</u>			
Sowing date	<0.001	0.217	0.71
<u>All genotypes – SD1-3 only[3]</u>			
Sowing date	<0.001	0.143	0.397
Genotype	<0.001	0.190	0.400
Sowing date * genotype	0.094	0.226	NS
Means within a sowing date		0.330	0.693

[1] – is no plants flowering while data in italic is from a genotype by sowing date combination that did not achieve 50% of plants flowering, thus was excluded from analysis.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

Appendix 11. Number of primordium produced per Haun Stage during the vegetative phase, where treatments achieved 50% of plants flowering, of four perennial ryegrass genotypes sown on five dates near Lincoln, New Zealand in the 2019/20 growing season.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	2.58	1.72	2.38	2.28	2.24
2. 17/04/2019	2.23	2.18	2.67	2.42	2.37
3. 14/06/2019	1.69	2.03	2.06	2.23	2.00
4. 30/08/2019	1.74	-[1]	-	-	-
5. 4/10/2019	2.29	-	-	-	-
Mean (SD1-3)	2.17	1.98	2.37	2.31	2.21
Mean (SD1-5)	2.11				

Parameter	P value	SEM	$LSD_{0.05}$
Medea only[2]	0.276	0.307	1.002
Sowing date			
All genotypes – Sow dates 1 – 3 only[3]			
Sowing date	0.356	0.161	0.633
Genotype	0.158	0.126	0.373
Sowing date * genotype	0.219	0.248	0.744
Means within a sowing date		0.217	0.646

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

Appendix 12. Rate of primodria production following between floral initiation and terminal spikelet of four perennial ryegrass genotypes sown on five dates aat Lincoln in the 2019/20 season.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	6.03	6.67	6.83	6.19	6.43
2. 17/04/2019	7.73	7.39	7.88	7.22	7.55
3. 14/06/2019	6.7	8.51	8.20	8.72	8.03
4. 30/08/2019	7.28	-[1]	-	-	-
5. 4/10/2019	4.62	-	-	-	-
Mean (SD1-3)	6.82	7.52	7.63	7.38	7.34
Mean (SD1-5)	6.47				
Parameter			P value	SEM	$LSD_{0.05}$
Medea only[2]			0.159	1.157	2.669
Sowing date					
All genotypes – SD1-3 only[3]					
Sowing date			0.013	0.211	0.827
Genotype			0.147	0.256	0.76
Sowing date * genotype			0.173	0.438	1.285
Means within a sowing date				0.443	1.316

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

Appendix 13. Number of primordium produced per Haun Stage during the vegetative phase, where treatments achieved 50% of plants flowering, of four perennial ryegrass genotypes sown on five dates near Lincoln, New Zealand in the 2019/20 growing season.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	43.2	48.2	44.6	43.8	44.9
2. 17/04/2019	34.3	42.7	38.6	39.3	38.7
3. 14/06/2019	25.9	36.0	32.0	34.2	32.0
4. 30/08/2019	24.3	-[1]	-	-	-
5. 4/10/2019	23.7	-	-	-	-
Mean (SD1-3)	34.5	42.3	38.4	39.1	38.6
Mean (SD1-5)	30.3				

Parameter	P value	SEM	$LSD_{0.05}$
Medea only[2]	<.001	0.49	1.59
Sowing date			
All genotypes – SD1-3 only[3]			
Sowing date	<.001	0.130	0.508
Genotype	<.001	0.454	1.349
Sowing date * genotype	0.008	0.693	2.051
Means within a sowing date		0.786	2.337

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

Appendix 14. Reproductive stem length of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	69.4	78.3	70.0	74.4	73.0
2. 17/04/2019	63.0	72.6	68.6	68.7	68.2
3. 14/06/2019	48.6	71.8	69.0	63.3	63.2
4. 30/08/2019	45.4	-[1]	-	-	45.4
5. 4/10/2019	38.1	-	-	-	38.1
Mean (SD1-3)	60.3	74.2	69.2	68.8	
Mean (SD1-5)	52.9				
Parameter			P value	SEM	$LSD_{0.05}$
Medea only[2] Sowing date			<.001	1.741	5.677
All genotypes – SD1–3 only[3]					
Sowing date			0.018	1.369	5.375
Genotype			<.001	1.141	3.39
Sowing date * genotype			0.003	2.192	6.538
Means within a sowing date				1.976	5.872

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

Appendix 15. Spikelet data

10.1.1 Spikelets per ear

The spiklelet number per ear was influenced by a sowing date by genotype interaction (P<0.05). Generally, the spikelet number reduced for all genotypes but the pattern of loss was different (Figure 10.1). For example, spikelets/ear was constant for 'Kleppe' between SD1 (28.4 ±0.76) and SD2 (27.6) but fewer were produced in SD3 (24.5). For 'Grasslands Impact', spikelets/ear was constant for SD2 (24.6) and SD3 (23.9) but both were lower than SD1 (27 spikelets/ear) (Table 10.1).

For 'Medea', the number of spikelets/ear reduced from 26.0 (±0.66) for SD1 to 13.4 for SD5 (Table 10.1).

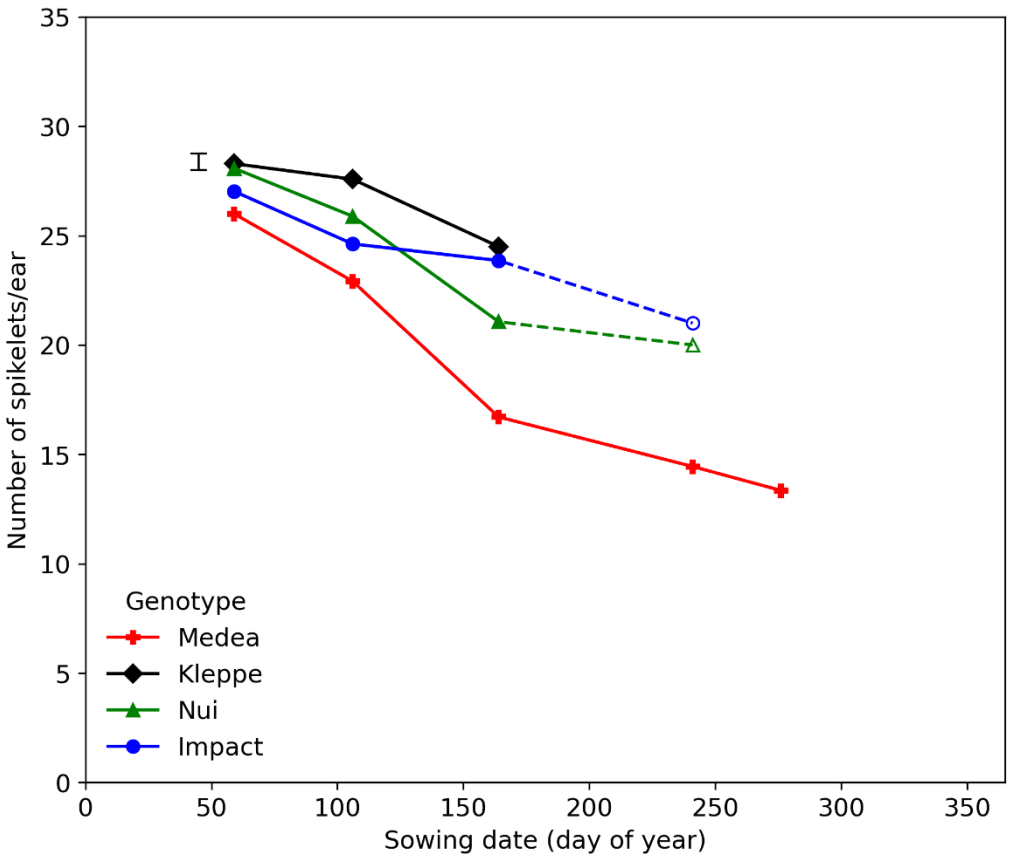


Figure 10.1. Number of spikelet's produced on the main stem of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand. Solid line is where the genotype reached 50% of plants flowering, dashed line shown for reference where less than 50% of plants flowered. Bar = standard error of the mean for the genotype by sowing date interaction.

Table 10.1. Number of spikelet's produced on the main stem of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand.

Sowing date	Genotype				Sowing date mean
	Medea	Kleppe	Grasslands Nui	Grasslands Impact	
1. 1/03/2019	26.0	28.4	28.1	27.0	27.4
2. 17/04/2019	22.9	27.6	25.9	24.6	25.3
3. 14/06/2019	16.7	24.5	21.1	23.9	21.5
4. 30/08/2019	15.3	-[1]	-	-	15.3
5. 4/10/2019	13.4	-	-	-	13.4
Mean (SD1-3)	21.9	26.81	25.01	25.17	24.7
Mean (SD1-5)	18.9				

Parameter	P value	SEM	$LSD_{0.05}$
Medea only[2]			
Sowing date	<0.001	0.661	2.157
All genotypes – SD1–3 only[3]			
Sowing date	<0.001	0.272	1.068
Genotype	<0.001	0.476	1.415
Sowing date * genotype	0.016	0.764	2.245
Means within a sowing date		0.825	2.451

[1] – genotype did not achieve 50% of plants flowering from this sowing date.

[2] Medea analysed alone across five sowing dates

[3] all genotypes analysed across sowing dates one to three to investigate genotype by sowing date interactions.

10.1.2 Spikelets relative to duration of initiation

The maximum number of spikelets/ear was consistent among genotypes for SD1 (Table 10.1). When plotted against the duration from FI^{HS} until TS^{HS}, the number of spikelets/ear was constant above a duration of 2.7 HS (±0.07) and reduced by 25/HS (±4.92) as the FI-TS duration reduced towards 2 HS (Figure 10.2).

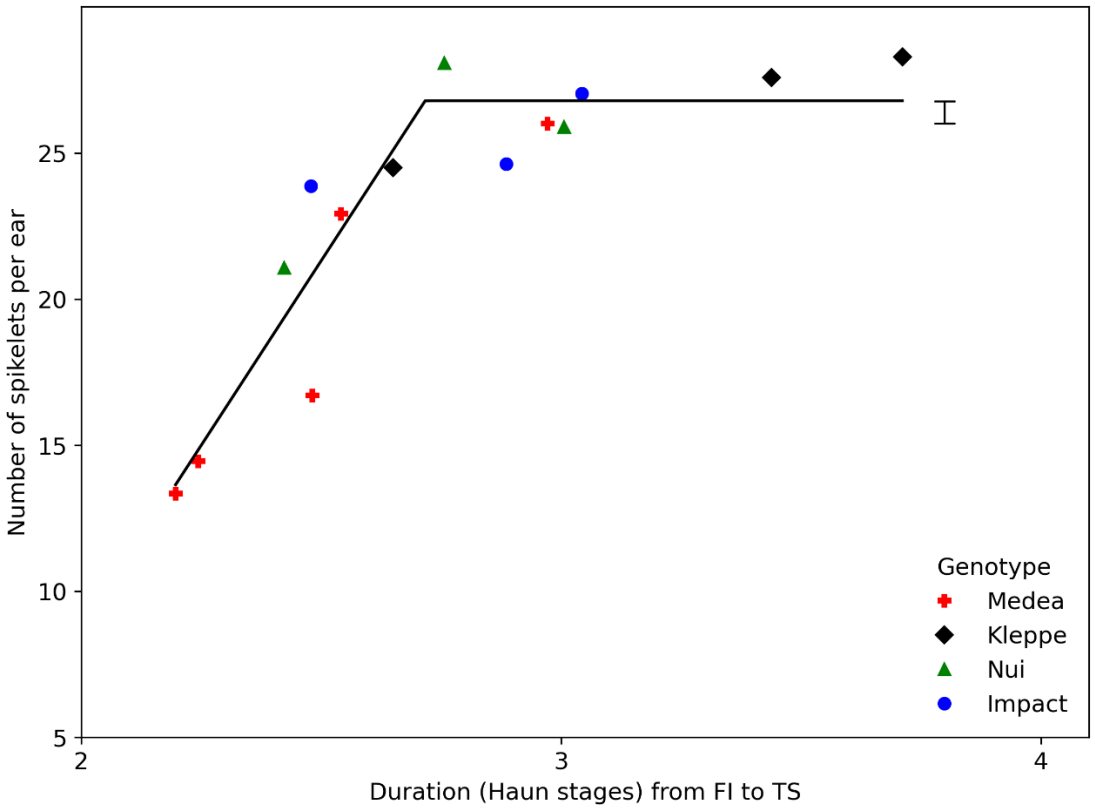


Figure 10.2 Number of spikelets per ear as influenced by the duration from floral initiation (FI) until terminal spikelet (TS) of four perennial ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand. Bar = standard error of the mean for the sowing date by genotype interaction (0.764). Lines = breakpoint x = 2.72 (±0.07), breakpoint y = 26.78 (±0.73), slope line 1 = 25.27 (±4.92), R^2 = 0.87.

10.1.3 Spikelets/ear and photoperiod

Since the FI-TS duration was influenced by Pp (Section 5.3.9), spikelet number was regressed against Pp at four developmental timings. All timings produced relationships that can estimate the number of spikelets/ear (Figure 10.3). For 'Medea' and 'Grasslands Impact' the reduction in the number of spikelets/h Pp (slope of the response) was not different between the stages of FI and TS, at ~2.3 for 'Medea' and ~1.4 for 'Grasslands Impact'. For 'Kleppe' the response was similar at FI and DR (mean 1.89) but increased at TS to 6.72, while 'Grasslands Nui' was intermediate (Figure 10.3). Physiologically, the Pp between DR and TS is likely to determine the end of the spikelet initiation period. Thus, the Pp between FI and TS, or their mean is allowed prediction of spikelet number for each genotype (Figure 10.3).

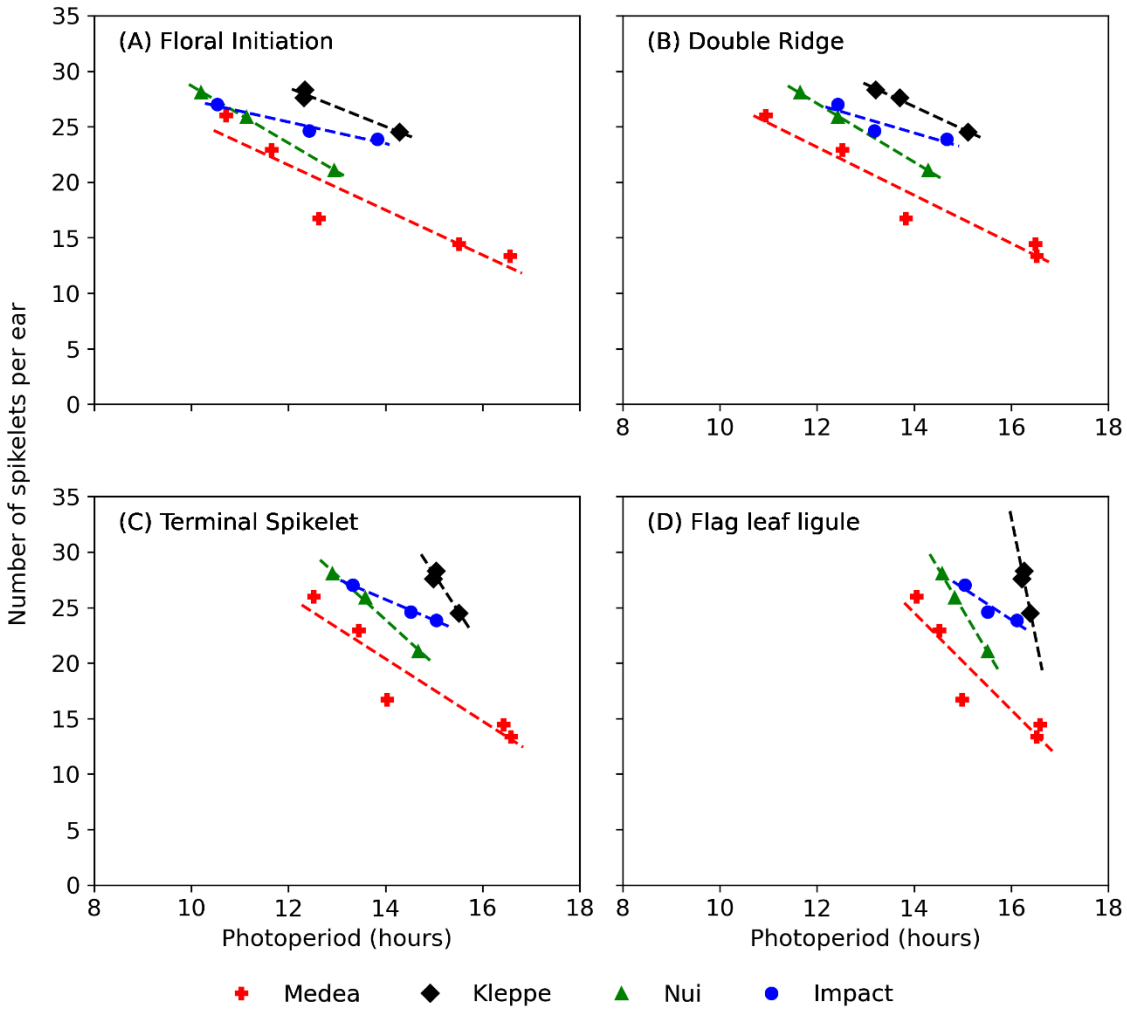


Figure 10.3. Number of spikelets/ear in response to photoperiod at four developmental timings of four perennial ryegrass genotypes when grown near Lincoln, New Zealand in the 2019/20 growing season.

Table 10.2. Linear regression parameters used to describe the number of spikelets/ear of four perennial ryegrass genotypes in response to photoperiod at four developmental timings of perennial ryegrass when grown near Lincoln, New Zealand in the 2019/20 growing season.

Genotype	timing	intercept	intercept se	Slope (spikelets/h Pp)	slope se	R^2	P value
Medea	FI	45.9	6.56	-2.03	0.48	0.81	0.02
	DR	49.2	4.82	-2.17	0.34	0.91	0.01
	TS	59.7	9.58	-2.81	0.65	0.81	0.02
	Final leaf	86.5	14.81	-4.43	0.97	0.83	0.02
Kleppe	FI	49.5	4.22	-1.75	0.32	0.93	0.12
	DR	55.3	2.32	-2.03	0.17	0.99	0.05
	TS	128.7	30.24	-6.72	1.99	0.84	0.18
	Final leaf	120.0	33.12	-5.84	2.07	0.78	0.22
Nui	FI	54.5	0.94	-2.58	0.08	1.00	0.02
	DR	58.8	0.73	-2.64	0.06	1.00	0.01
	TS	79.9	4.54	-4.00	0.33	0.99	0.05
	Final leaf	126.4	15.90	-6.82	1.07	0.95	0.10
Impact	FI	37.1	2.53	-0.98	0.21	0.92	0.13
	DR	42.4	8.91	-1.28	0.66	0.58	0.30
	TS	51.8	1.86	-1.86	0.13	0.99	0.04
	Final leaf	113.3	62.64	-5.76	4.09	0.33	0.39

Appendix 16. Parameters from linear regression analysis to describe the number of leaves produced post floral initiation in relation to photoperiod at six developmental timings of four ryegrass genotypes sown on five dates near Lincoln, Canterbury, New Zealand in the 2019/20 growing season.

Genotype	Timing	Intercept	Slope (leaves/h Pp)	R^2	P value	Saturating Pp (hours)
Medea	Sowing	3.06	0.108	0.240	0.667	8.70
	Emergence	3.06	0.108	0.240	0.667	8.70
	FI	12.5	-0.670	0.679	0.262	12.7
	DR	10.3	-0.454	0.769	0.221	14.0
	TS	16.6	-0.890	0.856	0.173	14.1
	Final leaf	24.8	-1.382	0.708	0.250	15.0
Kleppe	Sowing	2.09	0.298	0.049	0.484	6.39
	Emergence	2.09	0.298	0.049	0.484	6.39
	FI	15.6	-0.764	0.957	0.093	15.2
	DR	17.1	-0.811	0.681	0.262	16.1
	TS	76.4	-4.673	0.999	0.012	15.5
	Final leaf	40.5	-2.157	0.463	0.653	16.9
Nui	Sowing	2.18	0.252	0.516	0.327	7.23
	Emergence	2.18	0.252	0.516	0.327	7.23
	FI	10.1	-0.427	0.879	0.158	14.3
	DR	10.9	-0.443	0.925	0.124	15.5
	TS	14.2	-0.656	0.826	0.190	15.6
	Final leaf	21.8	-1.114	0.976	0.070	16.0
Impact	Sowing	2.82	0.227	0.950	0.101	5.19
	Emergence	2.82	0.227	0.950	0.101	5.19
	FI	9.13	-0.291	0.949	0.102	17.6
	DR	10.8	-0.388	0.654	0.273	17.5
	TS	13.4	-0.551	0.999	0.014	17.1
	Final leaf	13.2	-0.498	0.998	0.022	16.3