Thermal requirements for inflorescence emergence in plantain

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Abstract

Plantain (*Plantago lanceolata* L.) is increasingly utilised in New Zealand agricultural enterprises as part of a diverse pasture sward for livestock. Flowering behaviour is important in understanding plantain husbandry. The conversion of vegetative to reproductive growth alters the nutritional profile and yield, both of which may negatively impact on animal performance. Although plantain is described as a long day plant there is limited literature on environmental cues for inflorescence emergence (growth stage 51). The aims of this study were to a) quantify the thermal time (TT) requirements of inflorescence emergence between germplasm, noting any grouping trends, and b) determine the duration of inflorescence emergence within each germplasm. Sixty seeds of twenty different cultivars and germplasm of European origin were sown in spring and maintained in a glasshouse environment until they were transplanted to the field. All transplanted seedlings exhibited inflorescence emergence within 2350 °Cd or 145 days after sowing (DAS). Thermal time to inflorescence emergence was recorded for all twenty lines. There was a range of mean TT requirements for inflorescence emergence from 1246 to 1866 °Cd and 94 to 134 DAS. Thermal time to inflorescence emergence could further be divided into three groupings ($LSD_{5\%}$): 'early', 'mid' and 'late'. A broader duration of inflorescence emergence was positively correlated (R = 0.7300) with later flowering germplasm. Limitations of having a single site (mean annual temperature and precipitation is 11.8 °C and 635mm, respectively) and run over a single year make extrapolating data from this experiment throughout New Zealand difficult.

Introduction

Traditionally, New Zealand pastures were composed of two major species: perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). More recently, herbs such as plantain have become popular as part of a more diverse pasture agriculture due to the presence of secondary plant compounds (O'Connell *et al*., 2016), nutritional profile, drought tolerance (Stewart, 1996) and increasing animal intake (Gregorini *et al*., 2017). With a renewed interest in plantain for use in diverse pastures, there are currently eleven cultivars available in New Zealand.

Although plantain is common as a flat weed, the bred plantain cultivars are upright and vigorous. Plantain is typically sown as part of a pasture sward at a rate of 1-4 kg seed/ha (DairyNZ, 2013). Proprietary cultivars characteristically produce 10-15 t DM/ha/year as a monoculture with maximal growth rates of 80-140 kg DM/ha/day in late spring to early autumn (Powell *et al*., 2007).

Under vegetative conditions, plantain has a nutritional profile of 11-12 MJ/kg DM, 9-20% dry matter, 16-28% crude protein and 11-17% soluble sugar/starch (Harrington *et*

al., 2006; Sanderson *et al*., 2002; Cranston, 2014). Anthesis negatively affects both nutritional quality and biomass quantity by increasing the concentration of structural carbohydrates and fibre (Fraser and Rowarth, 1996).

Reproductive success is partially determined by the timing of flowering (Ream *et al*., 2014). In grasses induction of flowering tends to be coordinated with seasonal environmental cues, especially in temperate climates, notably winter temperature (vernalisation) followed by photoperiod (day length).

Plantain was described in 1948 as a long day plant (Snyder, 1948), where plants maintained under short day conditions remained vegetative for 14 months. Day length changes throughout the seasons, with increasingly marked variation further from the equator. Day and night lengths alter photoreceptor proteins in plants (cryptochrome or phytochrome) providing signalling for seasonal changes (Mauseth, 2003). Long day plants undergo induction of reproductive development when the night length falls below their critical photoperiod, thus they can be more accurately described as short night plants (Starr *et al*., 2013), with maximal inflorescence production occurring in continuous light. Long day plants tend to also have vernalisation requirements (Laurie and Macknight, 2004). However, it has been noted that plantain has a broad temperature range allowing growth and development in a wide variety of temperate habitats (Termura *et al*., 1981).

Plantain typically undergoes reproductive development from November through to March in Australasia (Moore *et al*., 2006) by way of small, inconspicuous hermaphroditic flowers arranged as inflorescences on axillary spikes (Clifford, 1962; Soekarjo, 1992). However, it also has adaptive plasticity to environmental conditions (Latzel *et al*., 2014) where inflorescence emergence can be altered by ambient temperature (Cavers *et al*., 1980). Plantain grown in warmer climatic regions flowers year round (Holm *et al*., 1977). Seedlings maintained under 16 hour days at 27 °C, 21 °C and 15 °C flowered in 59 days, 51 days and 130 days respectively (Stearns, 1955).

Thermal time requirements reflect plant growth in a cumulative, stepwise manner (Moot *et al*., 2000). Literature pertaining to TT accumulation for flowering in plantain is limited. Cardinal temperatures (base, minimum and maximum) in plantain is unestablished. Thermal time requirements for seed head emergence provides information for plant breeders in terms of developing early, mid and late flowering cultivars to coincide with livestock energy demands on farming enterprises. This information may also assist in determining sowing date for proprietary seed growers.

The objectives of this study were to quantify the TT requirements of inflorescence emergence between various plantain germplasm, thus allowing classifications of germplasms into groupings. A secondary aim was to determine the duration of inflorescence emergence within each germplasm line.

Materials and Methods

Experimental design

The trial was conducted at PGG Wrightson Seeds, Kimihia Research Centre, Lincoln, Canterbury, New Zealand (43°38'S, 172°28'E) during the spring-summer period. Sixty seeds from twenty plantain germplasm

were sown into seed raising soil mix in 60-cell propagation trays with punched drainage (1.25cm^3) on 17 August, 2017 (Day 0). Propagation trays were arranged in a randomised design in a tunnel house, receiving 10 mm/ha of irrigation twice daily. When 95% of seeds had germinated (Day 7), a HOBO® Temp/RH 3.5% Data Logger was set to record ambient temperature (° Celsius) at soil level every five minutes (288 records/day).

On day 42, 40 seedlings of each cultivar were randomly selected and transplanted into the field using a 1.25 cm^3 soil corer. The removed soil core was replaced with the plug plant, at a standard distance of 30 cm on the square. Seedlings were arranged in a complete randomised block design, consisting of four replicates with ten plants each. The Data Logger was relocated to the centre of the trial at the same time as transplanting, again at soil level.

Trial husbandry

Trial area was previously fallowed for twelve months. Ten days prior to transplanting, the final tilled trial area received 148 kg/ha of YaraCAN27% (calcium ammonium nitrate), equating to 40 kg nitrogen/ha; and sprayed with 5 L/ha of non-selective RoundUp Proactive 360 herbicide (360 g/L glyphosate). On day 81, 39 days after transplanting, the trial area was also sprayed with 0.3 L/ha of selective Gallant Ultra (520 g/L haloxyfop-P) to control *Poa annua* L.

Inflorescence emergence

All individual plants were observed daily between 1200-1300 hours for the presence of inflorescence emergence (axillary spikes). The date where three spikes were evident on a plant or 'inflorescence emergence' was recorded. Temperature records from the Data Logger were downloaded using the HOBO® software to Microsoft Excel. Thermal time records were correlated with date of inflorescence emergence. Accumulated TT was calculated using the equation: $TT = \sum(T_{day\ avg} - T_{base})$ where the mean daily temperatures were summed from germination (day 7) to inflorescence emergence of each plant.

A base temperature (T_b) of 4.0 °C was used due to a lack of literature on T_b in plantain. This is in line with other temperate legume and grass species which have T_b of ≤4.0 °C (Lonati *et al*., 2009; Moot *et al*., 2000), and another developmental plantain trial in New Zealand which used T_b of 5.0 °C (Powell *et al*., 2007). Temperatures remained above the assumed T_b throughout the trial. As the maximum mean daily temperature during the trial period was 27.4 °C, no temperatures were excluded from the calculations.

Statistical analysis

Data were processed using Microsoft Excel. One-way Analysis of Variance (ANOVA), Fisher's Least Significant Difference (LSD) and Linear Regression were carried out on all data with a significance level of $\alpha = 0.05$ using Genstat Version 19.

Results

Time to inflorescence emergence

Inflorescence emergence differed between cultivars (Figure 1; $p<0.001$; LSD = 79.14). Range of TT requirements varied from 1246 (Breeding line POR2) to 1866 °Cd (Boston). Corresponding DAS to inflorescence emergence also followed the same trend

(Figure 1; $p<0.001$; LSD = 3.770) with a range from 94 to 124 DAS. Statistically, there were three groupings ($LSD_{5\%}$) of TT requirements and DAS for inflorescence emergence. 'Early' flowering types, Breeding line DB33 to Breeding line POR2 (Figure 2), had an inflorescence emergence range of 1,246 – 1,427 °Cd and 94 – 103 DAS with a group mean of 1359 °Cd (SEM = 23.34) and 100 DAS (SEM = 1.115). The 'mid' flowering type consisted of only one germplasm line, Breeding line SG15, with a mean of 1554 °Cd (SEM = 28.07) and 108 DAS (SEM = 1.337). Lastly, inflorescence emergence of the 'late' grouping, Boston to Tuatara (Figure 1), ranged from 1,646 – 1,866 °Cd and 113-134 DAS, with a group mean of 1735 °Cd (SEM = 31.1) and 117 DAS (SEM = 1.488).

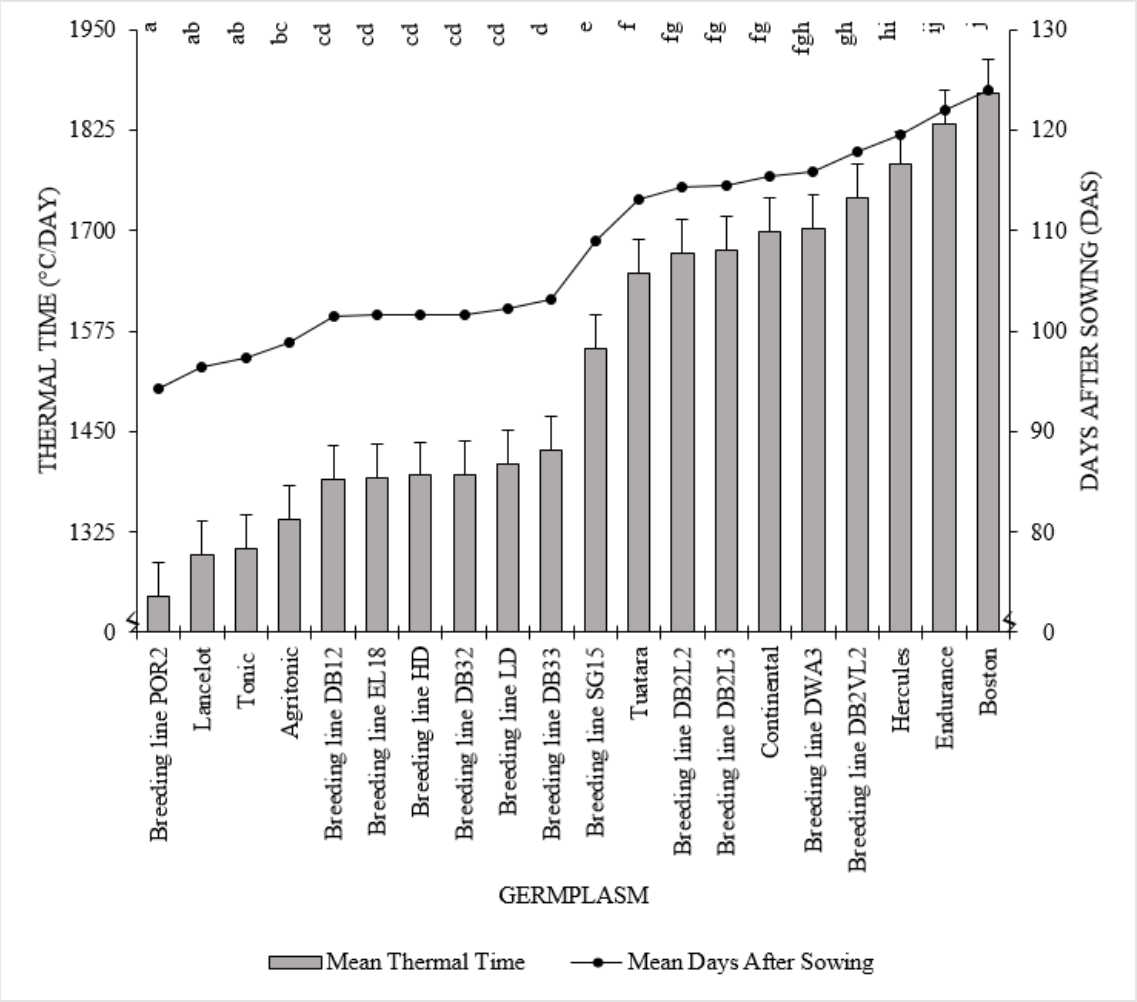


Figure 1: Comparison of thermal time requirement of three seed head emergence in *Plantago lanceolata* L. germplasm. Error bars represent half $LSD_{5\%}$, letters indicate post hoc groupings from Fisher's Least Significant Difference.

Duration of inflorescence emergence

Inflorescence emergence patterns of germplasm varied from tight flowering over a 19 day period for Tonic (Figure 2) to a broader flowering over 57 days for Boston. There is a positive linear relationship

between germplasm with longer TT requirements for inflorescence emergence and broader emergence duration (R = 0.7300). Commercialisation of a germplasm had no effect on the duration of inflorescence emergence ($p > 0.05$).

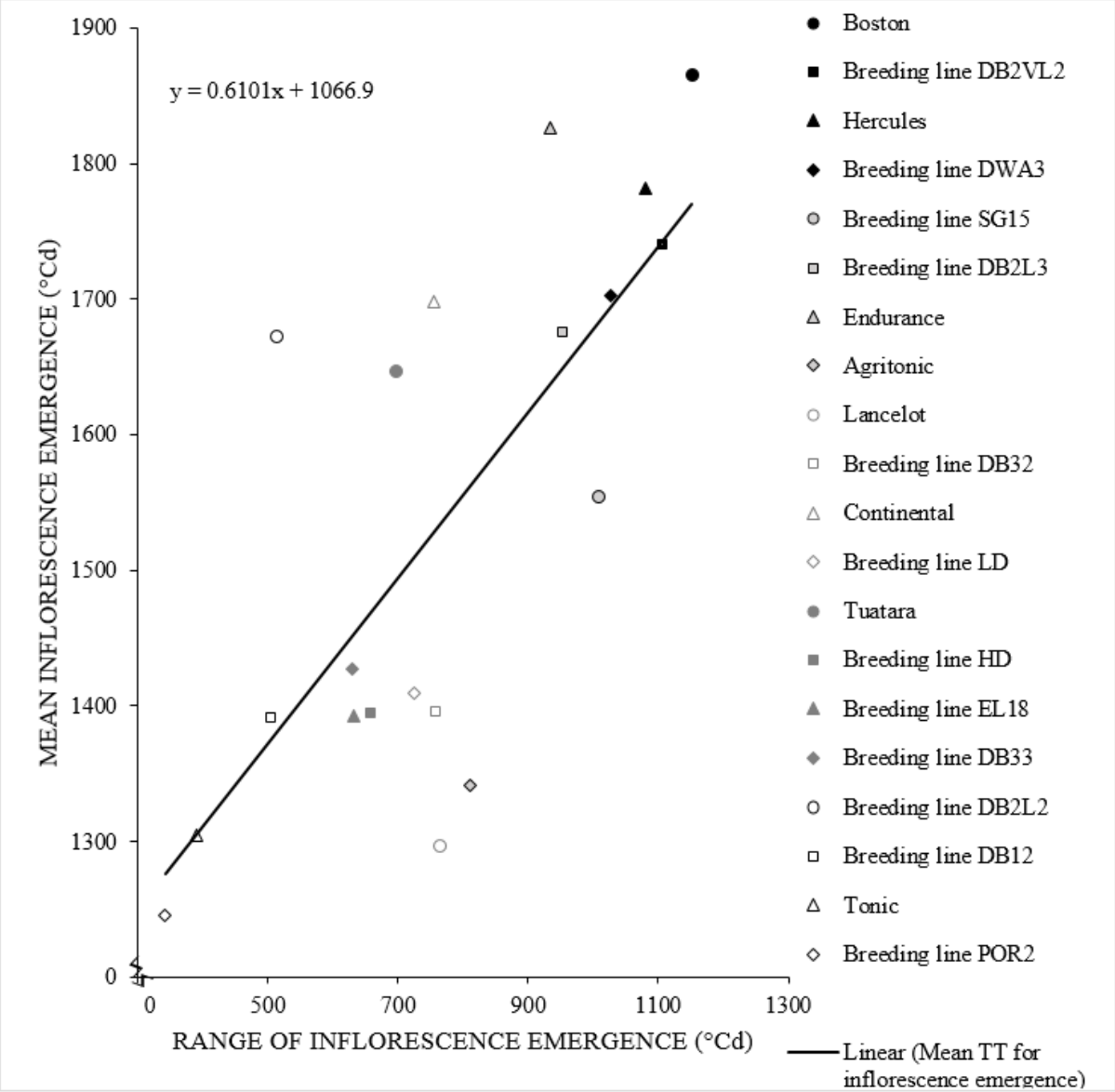
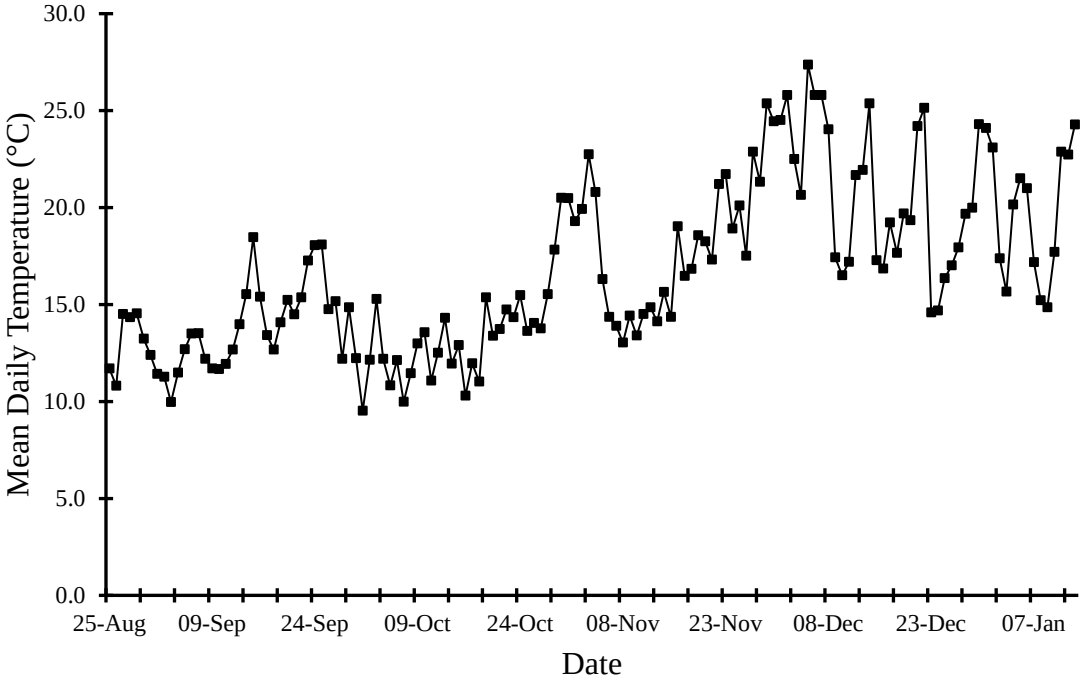


Figure 2: Correlation between duration of inflorescence emergence and mean inflorescence emergence for various *Plantago lanceolata* L. germplasm lines.

Environment

The trial site in Lincoln, Canterbury has mild, warm summers with moderate seasonality (Köppen-Geiger classification: Cfb). The mean annual temperature and precipitation is 11.8 °C and 635mm, respectively (Climate Data, 2018). Figure 3 reveals daily average temperatures during the 4 and a half month trial period at tray/soil level from Day 7.



*Inside glasshouse for first 42 days

Figure 3: Mean daily temperature (°C) recorded on a HOBO Data Logger at soil level located on the *Plantago lanceolata* inflorescence emergence trial site in Lincoln, Canterbury from 25 August 2017 to 13 January 2018.

Ambient temperatures recorded on the Data Logger for the trial period ranged from 7.9 to 40.6 °C (mean 16. 6 °C), equating to a mean TT of 13.9 °Cd. Maximal mean daily temperature during the trial period was 27.4℃. All 800 plants exhibited inflorescence emergence within 2350 °Cd or 145 DAS from a mid-August glasshouse sowing.

Discussion

Thermal time requirements of inflorescence emergence in plantain varied between germplasm. Statically, the germplasm in this experiment could further be grouped into early, mid and late flowering types (Figure 1). Some intermediate groups may also be evident with further research, particularly in the earliest and latest flowering germplasm. As environmental seasonal cues vary throughout geographical regions, flowering differences may be derived from the origins of the germplasm.

Breeding lines in the early flowering group have largely been bred out of 'Ceres Tonic' and 'Grasslands Lancelot', both of which are comparatively early flowering cultivars. Later flowering breeding lines were derived from germplasm material located in Northern European regions. Generalised groupings of this germplasm into an early-to-late continuum can allow farmers to select cultivars which better fit their enterprise goals and match livestock energy demands.

Plantain is a long day plant (Snyder, 1948), however flowering behaviour has been altered by ambient temperatures (Cavers *et al*., 1980; Stearns, 1955).

Differences in inflorescence emergence between germplasm supports a requirement for TT accumulation. It is likely there is an interaction between day length and TT accumulation.

Photoperiod requirements are often associated with a vernalisation requirement (Ream *et al*., 2014). There is little literature on temperature requirements for vernalisation in plantain. Vernalisation in other temperate plant species was found to be between 3 and 12 °C (Porker *et al*., 2016). With ambient temperature remaining above 7.9 °C throughout the trial period, autumn sown plantain appears to have limited vernalisation requirements in this climate. As germplasm of commercial cultivars have been found in numerous temperate origins (Woldendorp, 1992), potential differences in induction requiring a vernalisation period is likely to be dependent on the origin of the germplasm. No extrapolations can be made as to whether inflorescence emergence could be influenced by a chilling period. More research is required to determine if there is an obligate or facultative temperature requirement by maintaining higher temperatures throughout the trial period verse a control maintained outdoors.

The range of flowering within germplasm lines of 19 – 57 days is consistent with Rumball *et al*., (1997) who found 'Grasslands Lancelot' had a flowering range of 45 days from November to January. Plantain germplasm has a greater flowering range compared to perennial ryegrass (Anslow, 1963; Wilkins, 1991), representing an area for improvement in breeding (Jung and Muller, 2009).

Germplasm which required longer TT requirements for inflorescence emergence tended to have a greater flowering duration. The basis for this relationship is unknown, but likely causes may be a) change in environmental conditions, such as soil moisture, and increasing mean daily temperature (Figure 3); or b) a result of plant breeding to change the flowering date from the original earlier commercial cultivars 'Ceres Tonic' and 'Grasslands Lancelot'. As plantain is a perennial plant, these effects of inflorescence emergence may be overcome in subsequent years.

Inflorescence emergence was measured in both TT and days after sowing. However, crop maturity is referred to by estimated days after sowing rather than TT accumulation, due to the relative difficulty of measuring TT. Therefore, estimated days after sowing to inflorescence emergence is likely to be a more practical tool than TT accumulation.

Using DAS to predict inflorescence emergence can allow changes in grazing management to suppress reproductive development. Plantain in a vegetative state has a higher nutritional quality and biomass quantity than a reproductive state (Fraser and Rowarth, 1996). Data from this trial suggests that defoliation prior to 109 DAS for a mid-flowering type in Canterbury with similar ambient temperatures to this trial period is likely to maintain the plant in a vegetative state for a longer period.

However, recommendations in grazing management from this data set are limited. Firstly, the adaptive plasticity of plantain in terms of initiation of flowering under various environmental conditions needs to be established. Secondly, as TT requirements were collected at a single site in a single year, there is some difficulty in inferring the DAS measurements to alternative sites or between years. This is likely to be of particular importance to sites which do not have mean

daily TT of 13.9 °Cd during spring and summer. More research to determine inflorescence emergence between sites and years is required before grazing recommendations can be made.

Conclusions

Inflorescence emergence in plantain appears to be related to TT requirements during periods of long days and short nights. The mean TT requirements for inflorescence emergence ranged from 1246 to 1866 °Cd and 94 to 134 DAS. Using $LSD_{5\%}$ germplasm can be defined as being either early (1,246 – 1,427 °Cd, 94 – 103 DAS); mid (1,428 °Cd, or 109 DAS); or late flowering types (1,646 – 1,866 °Cd, or 113-134 DAS). Germplasm requiring more TT accumulation for inflorescence emergence was positively correlated with a broader inflorescence emergence range (R = 0.7300). As the conversion of vegetative growth to reproductive growth alters nutritional profile and yield, knowledge of timing of inflorescence emergence is likely to be important in plant management, cultivar selection in different locations, plant breeding, and seed production. This trial was conducted under in an environment where the temperature ranged from 7.9 to 40.6 °C (mean 16. 6 °C) throughout spring and summer. However, more research is required in determining the TT and DAS for inflorescence emergence between sites and years, along with the relationship between heritability of inflorescence emergence based on geographically distinct groupings.

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