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Comparison of two methods for estimating thermal requirements for germination of three turfgrass species at suboptimal temperatures

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ABSTRACT

Temperature is one of the most influential environmental factors for germination. We compared germination response to suboptimal temperature for the turfgrass species red fescue (*Festuca rubra* ssp. *litoralis*), perennial ryegrass (*Lolium perenne*), and Kentucky bluegrass (*Poa pratensis*), using two different approaches for data analysis. Two cultivars of each species were germinated at five constant temperatures (8 to 24°C), and germination was recorded one to three times per day. For each temperature, cultivar, and species, cumulative germination curves were fitted by the Weibull function. Predicted germination rates for the nine germination deciles were regressed against temperature, and the base temperature (T_b) for germination did not differ between deciles. Thus, assuming a constant T_b for all seeds in a population, cumulative germination data were analysed by two population-based methods, viz. the repeated probit analysis and a non-linear regression method, which integrates the thermal time model in the Weibull function. In most cases, the two methods resulted in similar estimates of T_b and reasonable estimates of thermal time to 50% germination ($\theta_T(50)$). The repeated probit analysis, however, described poorly the variation in $\theta_T(50)$, whereas the non-linear regression method provided a good description of the skewed distribution of $\theta_T(50)$. The results demonstrate that the repeated probit analysis provides an insufficient description of germination response to temperature for whole seed populations. The non-linear regression method should, therefore, be used when studying germination response to temperature for whole populations and when thermal time to germination is skewed.

Key words: *Festuca rubra*, germination models, *Lolium perenne*, non-linear regression, *Poa pratensis*, repeated probit analysis, temperature, thermal time, Weibull function

INTRODUCTION

Temperature is one of the most important factors for germination and affects both the timing of germination and the final germination percentage (Bewley and Black, 1994). At suboptimal temperatures, germination rate, i.e. the reciprocal time to germination, often increases linearly with germination temperature (e.g. Hegarthy, 1973; Bierhuizen and Wagenvoort, 1974), although the relationship may be non-linear in certain cases (Marshall and Squire, 1996). In case of a linear relationship, germination response to suboptimal temperature can often be described by a thermal time model, which assumes that in order to germinate, a seed requires accumulation of a certain thermal time or heat sum, θ_T, above a minimum base temperature, T_b, under which germination rate is theoretically zero (Bierhuizen and Wagenvoort, 1974; Garcia-Huidobro *et al.*, 1982; Yeh and Atherton, 2000).

Within a population of seeds, T_b is often constant for all seeds (Covell *et al.*, 1986; Yeh and Atherton, 2000) although it may vary more or less (Trudgill *et al.*, 2000) and sometimes follows a normal distribution (Washitani and Takenaka, 1984). On the other hand, θ_T generally varies among seeds within a population (Garcia-Huidobro *et al.*, 1982; Washitani and Takenaka, 1984) and has been found to be normally distributed (Ellis *et al.*, 1986; Covell *et al.*, 1986) or log-normally distributed (Covell *et al.*, 1986; Dahal *et al.*, 1990). Consequently, a separate regression line is needed for individual germination percentages to describe the relationship between temperature and germination rate.

Three problematic circumstances arise when analysing germination responses to temperature. Firstly, from a statistical perspective it is unfavourable to analyse data in two steps, i.e. by first estimating the time to achieve a certain germination percentile, and then use the estimate in a subsequent regression procedure (Covell *et al.*, 1986). Data should preferably be analysed in one step, using the original data. Secondly, it may be hazardous to extrapolate beyond the observed temperature range, which is often done to estimate T_b (Phelps and Finch-Savage, 1997). Thirdly, it is desirable to describe germination behaviour for whole populations rather than for specific germination percentiles only. To solve these problems, Covell *et al.* (1986) suggested the repeated probit analysis in which a single equation describes the influence of suboptimal temperature on germination rates for a whole population. The model assumes a constant T_b and a normally or log-normally distributed θ_T, and the model has been used successfully for a number of species (Covell *et al.*, 1986; Ellis *et al.*, 1986; Dahal *et al.*, 1990).

The distribution of germination times in a seed population is, however, often skewed (Bradford, 1995). Although a positively skewed distribution may be transformed to a normal distribution by logarithmic transformation (Scott *et al.*, 1984), this may not be sufficient to describe germination data adequately. The Weibull function has demonstrated the ability to take into account the potential skewness when describing cumulative germination data (Brown and Mayer, 1988; Dumur *et al.*, 1990). In this study, we suggest a non-linear regression method based on the Weibull function for estimating T_b and the distribution of θ_T on a population basis. The method is more flexible than the repeated probit method when describing skewed germination data.

The temperate grass species slender creeping red fescue (subsequently just termed red fescue) (*Festuca rubra* L. ssp. *litoralis* Vasey), perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poa pratensis* L.) are commonly sown in mixture for turfgrass on sports fields. To understand the establishment success of these species when sown together under various environmental conditions, we studied their individual response to suboptimal temperatures, which are generally encountered in the seedbed. The purpose of the study was 1) to test if T_b varied within and between seed populations of red fescue, perennial ryegrass, and Kentucky bluegrass, 2) to compare the ability of the repeated probit method and a more flexible non-linear regression method to describe germination response to temperature for the three species.

MATERIALS AND METHODS

Seed material

Six seed lots were included in the study. Red fescue was represented by one seed lot of each of the two cultivars Cinderella and Symphony, whereas perennial ryegrass was represented by the cultivars Figaro and Taya and Kentucky bluegrass by the cultivars Andante and Broadway. All seed lots were commercially produced and harvested in Denmark in July 2000 and delivered by DLF Trifolium, Denmark. Seeds were stored at 5°C from harvest until germination experiments were carried out from March to May 2001, and primary dormancy was expected to have disappeared during the storage period.

Germination experiment

Each of the six seed lots were germinated at constant temperatures of 8, 12, 16, 20, and 24°C with 12/12 hours light/darkness per day. Seeds were germinated on top of paper in a "mini Jacobsen apparatus" (Copenhagen Tank), and each combination of seed lot and temperature was represented by four replicates of 100 seeds. Prior to the germination test, the filter paper was soaked in a 0.2% solution of KNO_3 (ISTA, 1996). The germination boxes were placed in five germination incubators (Termaks KBP 2324V, Norway), which were adjusted to the five different temperatures. The germination boxes were daily rearranged within the incubators to avoid effects of potential temperature gradients within the incubators.

Germination was recorded one, two or three times per day depending on germination activity, and the exact time from the start of the experiment to the inspection was recorded. A seed was regarded as germinated when the radicle had protruded at least 1 mm. Only seeds with normal radicles were counted. Germinated seeds were removed from the germination test. Germination tests were terminated when no new germination was observed within the four replicates for three consecutive days.

Analysis of germination data

For data analysis, mean germination time courses were obtained for each combination of temperature, cultivar, and species by calculating the mean cumulative germination percentages of the four replicates at each counting time. Observations with no further increase in germination percentage during the rest of the germination test do not contribute with information, and these observations were excluded from the data set before analysis.

Analysis of the germination response to suboptimal temperature was based on the thermal time model (Bierhuizen and Wagenvoort, 1974; Garcia-Huidobro *et al.*, 1982). The model states that thermal time to germination $\theta_T(g)$ (°days) of percentile g can be calculated as

$$\theta_T(g) = (T - T_b)^* t_g \quad [1]$$

where T is the actual temperature, T_b is the base temperature for germination below which germination does not occur, and t_g is the actual time to germination of percentile g. Equation [1] can be rearranged:

$$1/t_g = (T - T_b)/\ \theta_T(g) \quad [2]$$

where $1/t_g$ is the germination rate which is linearly related to temperature T with the slope $1/\theta_T(g)$, i.e. the slope is specific for each germination percentile g, and T_b is the intercept with the x-axis.

The data analysis initially included a test of effect of temperature on final germination percentage and a test of differences in T_b between different germination percentiles and between cultivars and species by linear regression. Since there was no indication of significant differences in T_b between seeds within a population, the parameters T_b and $\theta_T(g)$ were then estimated by two different methods, both assuming a common T_b for all seeds in a population, viz. by repeated probit analysis and by non-linear regression analysis.

Test of effect of temperature on final germination percentage: If final germination percentage is affected by suboptimal temperature, this may indicate that T_b varies within seed lots, provided the germination test is not terminated before germination has finished. The effect of temperature on final germination percentage was therefore tested in a model, which included species, cultivar nested within species, and temperature as explanatory class variables and final germination percentage as response variable. The final germination percentages were logit-transformed to obtain homogeneous variance, and final germination percentages within a cultivar were compared by pairwise t-tests, using the variation between replicates as residual variance. The test was carried out using the glm procedure of the SAS package (SAS, 2000).

Test of variation in T_b by linear regression: The test of differences in T_b between germination percentiles was a two-step method which corresponds to the methodology applied by e.g. Garcia-Huidobro *et al.* (1982) and Marshall and Squire (1996). In step one, t_g was estimated for various levels of g and at each germination temperature, and in step two, T_b was estimated by linear regression, using estimates from step one.

In step one, each of the observed cumulative germination time courses was analysed, using the nlmixed procedure of the SAS package (SAS, 2000). For all species, cultivars, and temperatures, the Weibull function was fitted to the relationship between the germination percentile g and time t (days) according to Brown and Mayer (1988):

$$g = m^*[1\text{-}\exp(-(k^*(t\text{-}z))^c)]^*100 \qquad [3]$$

where 100^*m is the asymptotic final germination percentage, k is the rate of increase in germination percentage, z is the lag time before germination commences, and c is a shape parameter. Values of t_g, based on the number of viable seeds, were predicted from each of the estimated Weibull functions by rearranging equation [3]:

$$t_g = z + (1/k)^*[\ln(100/(100\text{-}g))]^{(1/c)} \qquad [4]$$

For each species and cultivar, t_g was calculated for nine germination deciles from 10 to 90%.

In step two, T_b was estimated according to equation [2] by linear regression of the reciprocal of the t_g values from the estimated Weibull functions against germination temperature, using an iterative routine which optimised the T_b for each decile. Since the constraint of equation [2] is on the x-axis, the analysis was carried out using the nlmixed procedure of the SAS package (SAS, 2000), although the model is linear. The procedure produces results equivalent to those of the glm procedure but allows test of differences and estimation of standard errors of estimates of T_b. The analysis included data for the nine germination deciles for all cultivars and species, and difference in T_b was tested between germination deciles, between cultivars, and between species.

Repeated probit analysis: The repeated probit analysis, as described by Covell *et al.* (1986), Ellis *et al.* (1986), Dahal *et al.* (1990), is a one-step method which provides estimates of T_b and $\theta_T(g)$ by analysis of the raw data. The model assumes a constant T_b and a normal or log-normal distribution of $\theta_T(g)$ within the seed population. The analysis was done using the probit procedure of the SAS package (SAS, 2000). All observed germination percentages on probit scale were regressed against ln thermal times, $\ln\theta_T(g)$, to germination of percentile g, varying the value of T_b stepwise by 0.01°C until the best fit was obtained, according to the equation:

$$\text{probit}\ (g) = \{\ln[(T\text{-}T_b)^*t_g] - \ln[\theta_T(50)]\}/\sigma_{\theta T} \qquad [5]$$

where probit (g) is the probit transformation of the cumulative germination percentage g, $\theta_T(50)$ is the thermal time to 50% germination, and $\sigma_{\theta T}$ is the standard deviation of $\ln\theta_T$ among individual seeds in the population (Dahal *et al.*, 1990). Since the germination percentage was in some cases considerably below 100%, the analysis was based on the proportion of viable seeds, determined by the maximum final germination percentage obtained within a seed lot. The analysis was carried out separately for each cultivar and species. Once T_b was estimated, all times to germination, t_g, were normalized on a thermal time scale by multiplying by the factor $(T\text{-}T_b)$ (Garcia-Huidobro *et al.*, 1982).

Non-linear regression analysis: Like the repeated probit analysis, the non-linear regression analysis is a one-step method in which all parameters are estimated in an analysis based directly on the observed data. The analysis was based on an equation which integrates the thermal time model (equation [1]) and the Weibull function (equation [3]):

$$g = m^*[1\text{-}\exp(\text{-}(k^*[(T\text{-}T_b)^*t\text{-}z])^c)]^*100 \qquad [6]$$

where g is germination percentage, $(T\text{-}T_b)^*t$ is thermal time, and where the constant parameters T_b, m, k, z, and c were estimated in the non-linear regression procedure. Thus, T_b was optimised until the least variation in thermal time to germination was obtained for observations from the different germination temperatures. The equation estimates progress in cumulative germination on a thermal time scale, and the thermal time $\theta_T(g)$ to obtain germination of percentile g can be predicted in a similar way as in equation [4]. The analysis was carried out separately for each cultivar and species, using the nlmixed procedure of the SAS package (SAS, 2000), which allowed estimates of standard errors of all parameter estimates.

RESULTS

Observed germination pattern and final germination percentage

Temperature had a marked effect on the timing of germination of all species and cultivars with a reduced time to germination when temperature increased from 8°C to 24°C (Fig. 1). Kentucky bluegrass germinated more slowly than red fescue and perennial ryegrass at all five temperatures. The effect of temperature on final germination percentage was less pronounced (Fig. 1). In

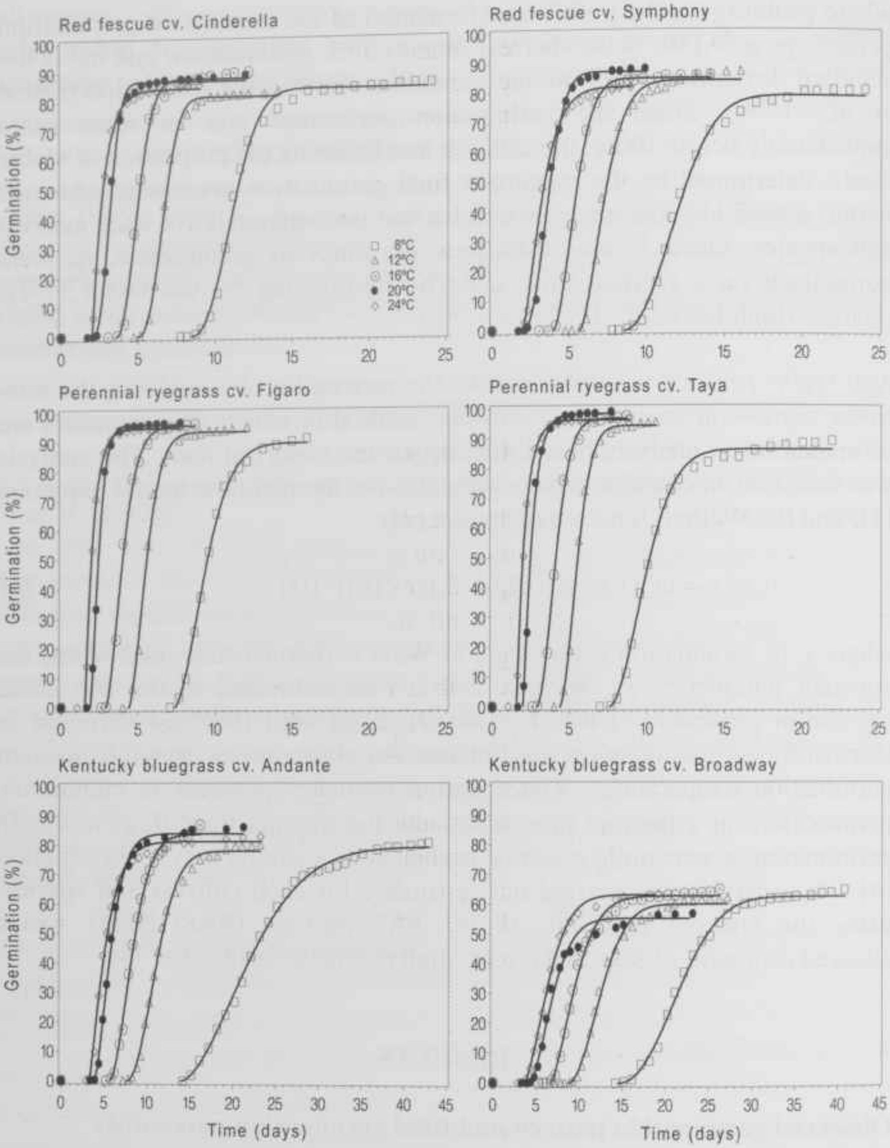

Figure 1. Germination time courses for germination of two cultivars of red fescue, perennial ryegrass, and Kentucky bluegrass, respectively, at five constant temperatures. Symbols indicate observed germination and full lines indicate the time courses predicted by the Weibull function, fitted to individual curves. Note the different time scale for Kentucky bluegrass cultivars.

red fescue, final germination percentage ranged from 83.0 to 89.8% and from 81.0 to 88.0% for cv. Cinderella and cv. Symphony, respectively, with no significant differences between temperatures within a cultivar ($P=0.060$ and $P=0.123$, respectively). In perennial ryegrass, final germination percentage ranged from 91.3 to 96.8% in cv. Figaro and from 89.3 to 99.0% in cv. Taya. In both cultivars, final germination percentage was lower at 8°C than at 20°C ($P=0.003$ and $P<0.001$, respectively) with no differences between temperatures above 8°C. In Kentucky bluegrass cv. Andante, final germination ranged from 80.3 to 87.0% and in cv. Broadway from 57.0 to 65.3%, and there was no significant difference between temperatures ($P=0.158$ and $P=0.326$, respectively).

Variation in T_b estimated by linear regression

Individual cumulative germination curves for each temperature were described by the Weibull function within all six seed lots (Fig. 1). There was a linear relationship between $1/t_g$ and temperature with a positive slope for all germination deciles, cultivars, and species ($P<0.001$) with decreasing slope with increasing decile (Fig. 2). Within all cultivars and species, the regression model could be reduced to a model with a common T_b for all germination deciles, indicating that T_b is constant for seeds within a population (Table 1). Within red fescue and perennial ryegrass, T_b was not significantly different for the two cultivars, whereas Kentucky bluegrass cv. Andante had a higher T_b than cv. Broadway (Table 1). T_b differed between the three species ($P<0.001$) with a higher T_b for perennial ryegrass than for red fescue ($P<0.001$) and a lower T_b for Kentucky bluegrass cv. Broadway than for red fescue ($P=0.025$). The T_b of Kentucky bluegrass cv. Andante was similar to those of the red fescue cultivars ($P=0.999$).

Repeated probit analysis

Within each cultivar and species, germination data for all temperatures were fitted by repeated probit analysis, resulting in estimates of T_b, $\ln\theta_T(50)$, and $\sigma_{\theta T}$ and derived estimates (Table 2). The germination data were normalized on a thermal time scale, using the estimated T_b (Fig. 3). The observations from each germination temperature converged reasonably well into a single germination course on the thermal time scale, except for Kentucky bluegrass cv. Broadway where there was a larger variation in thermal time at a given germination percentile. The repeated probit analysis

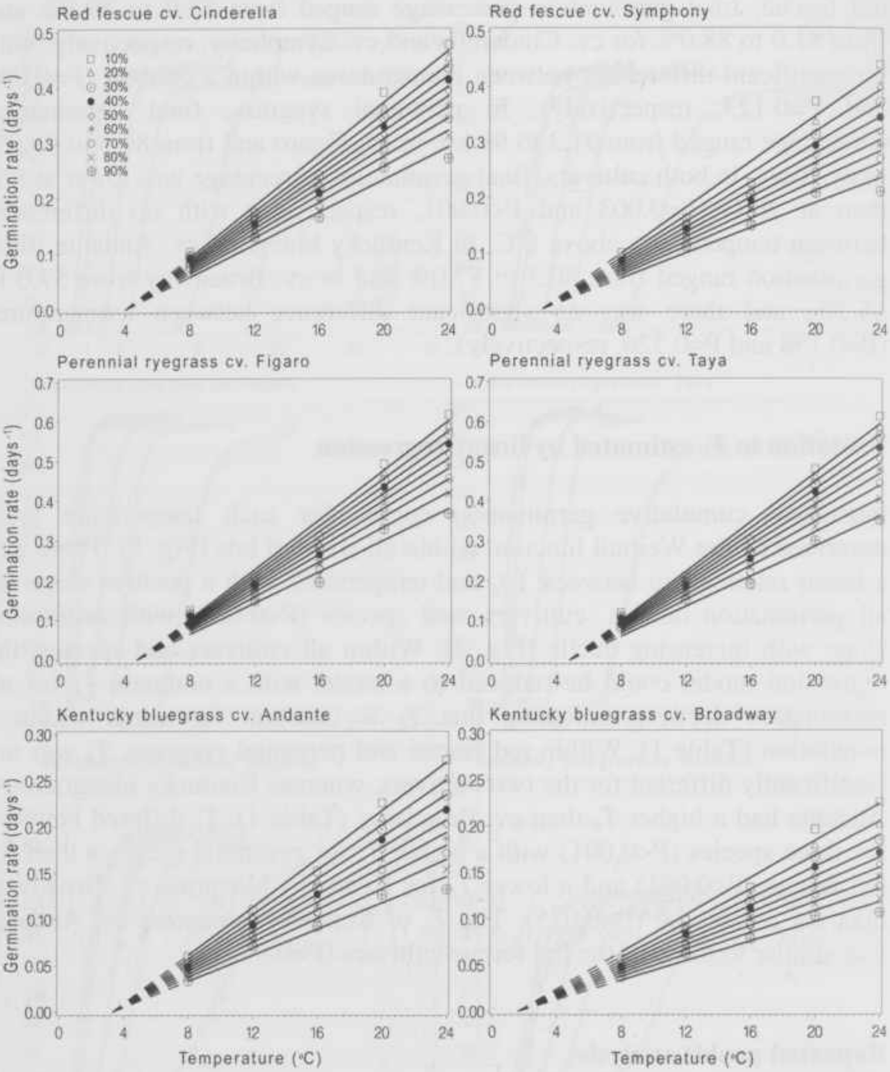

***Figure** 2. Relationship between germination rate, i.e. the reciprocal of time to germination percentage, and suboptimal germination temperature for two cultivars of each of the species red fescue, perennial ryegrass, and Kentucky bluegrass, respectively. Symbols indicate germination rates for various germination deciles as estimated by the Weibull function. Full lines indicate regression lines constrained to a common base temperature for germination within each cultivar, and broken lines indicate regression lines extrapolated to the intercept with the x-axis, which is an estimate of the base temperature for germination. Note the different scale on the y-axis.*

Table 1. *Estimates (±SE) of base temperature for germination (T_b) of two cultivars of red fescue, perennial ryegrass, and Kentucky bluegrass, respectively, determined by linear regression of germination rate for nine germination deciles against germination temperature. T_b indicates the best common estimate for the nine germination deciles within a cultivar, and the best common estimate for two cultivars within a species, respectively. P_{Tb} indicates difference in T_b between germination deciles within cultivars and between cultivars within species.*

Species	Cultivar	T_b	P_{Tb}
		(°C)	
Red fescue	Cinderella	3.95 ± 0.30	0.789
	Symphony	3.12 ± 0.33	0.175
	Cinderella + Symphony	3.60 ± 0.23	0.069
Perennial ryegrass	Figaro	4.70 ± 0.29	0.997
	Taya	4.79 ± 0.29	0.974
	Figaro + Taya	4.74 ± 0.21	0.752
Kentucky bluegrass	Andante	3.32 ± 0.23	0.558
	Broadway	1.63 ± 0.35	0.112
	Andante + Broadway	2.67 ± 0.21	<0.001

predicted the highest final germination percentage exactly since the analysis was adjusted to the proportion of viable seeds in the seed lots. The estimated time course, however, generally provided a poor prediction of the pattern in cumulative germination over time (Fig. 3). The time to reach a germination percentage around 50% was well predicted by the repeated probit analysis, whereas times to lower and higher percentages were poorly predicted with too large estimates of $\sigma_{\theta T}$ and the derived $\theta_T(25\text{-}75)$.

Plots of the probit regression (not shown) indicated that especially observations of high germination percentages deviated systematically from the predicted regression line. The repeated probit regression was, therefore, also done after exclusion of observations exceeding 90% and 85% of final germination percentage, respectively. Exclusion of observations above 90% improved the fit of the probit analysis considerably except for Kentucky bluegrass cv. Broadway, and exclusion of observations above 85% improved the fit further for all species and cultivars (data not shown). The estimates of T_b and $\ln\theta_T(50)$ only changed very little by the exclusion whereas $\sigma_{\theta T}$ was reduced by up to 50% compared to the estimates of Table 2, except for Kentucky bluegrass cv. Broadway.

Table 2. *Estimates of thermal requirements for germination of two cultivars of red fescue, perennial ryegrass, and Kentucky bluegrass, respectively, determined by the repeated probit analysis method.* T_b *is the base temperature for germination,* $\ln\theta_T(50)$ *is the ln-transformed thermal time to 50% of final germination,* $\sigma_{\theta T}$ *is the standard deviation in thermal time.* $\theta_T(50)$ *and* θ_T *(25-75) are derived estimates of thermal time to 50% of final germination and thermal time from 25 to 75% of final germination, respectively, on a normal thermal time scale.* R^2 *is the coefficient of determination of the regression of probit germination against ln thermal time to germination.*

Species	Cultivar	T_b (°C)	$\ln\theta_T(50)$ (ln °days)	$\sigma_{\theta T}$ (ln °days)	$\theta_T(50)$ (°days)	$\theta_T(25\text{-}75)$ (°days)	R^2
Red fescue	Cinderella	2.68	4.16	0.31	63.9	26.7	0.76
	Symphony	2.49	4.28	0.31	72.2	30.2	0.82
Perennial ryegrass	Figaro	3.31	3.83	0.27	46.2	16.6	0.77
	Taya	3.98	3.80	0.31	44.9	19.1	0.73
Kentucky bluegrass	Andante	3.24	4.69	0.35	108.4	52.4	0.75
	Broadway	0.46	5.08	0.34	160.2	73.9	0.83

Non-linear regression analysis

The non-linear regression analysis provided estimates of T_b and the Weibull parameters m, k, z, and c as well as the derived parameters $\theta_T(50)$ and $\theta_T(25\text{-}75)$ (Table 3). The estimated T_b values were used to normalize cumulative germination on a thermal time scale (Fig. 3). Due to the similarity between the T_b estimates from the repeated probit analysis (Table 2) and the non-linear regression analysis (Table 3), the observations converged into a similar curve for the two methods. An exception from this was Kentucky bluegrass cv. Broadway where the higher T_b estimate of the non-linear regression analysis resulted in a lower variation in thermal time to obtain a given germination percentile.

The germination time course estimated by the non-linear regression analysis provided a good prediction over the whole range of the observed time course, and the prediction was clearly superior to that of the repeated probit analysis (Fig. 3). The predicted final germination percentage was

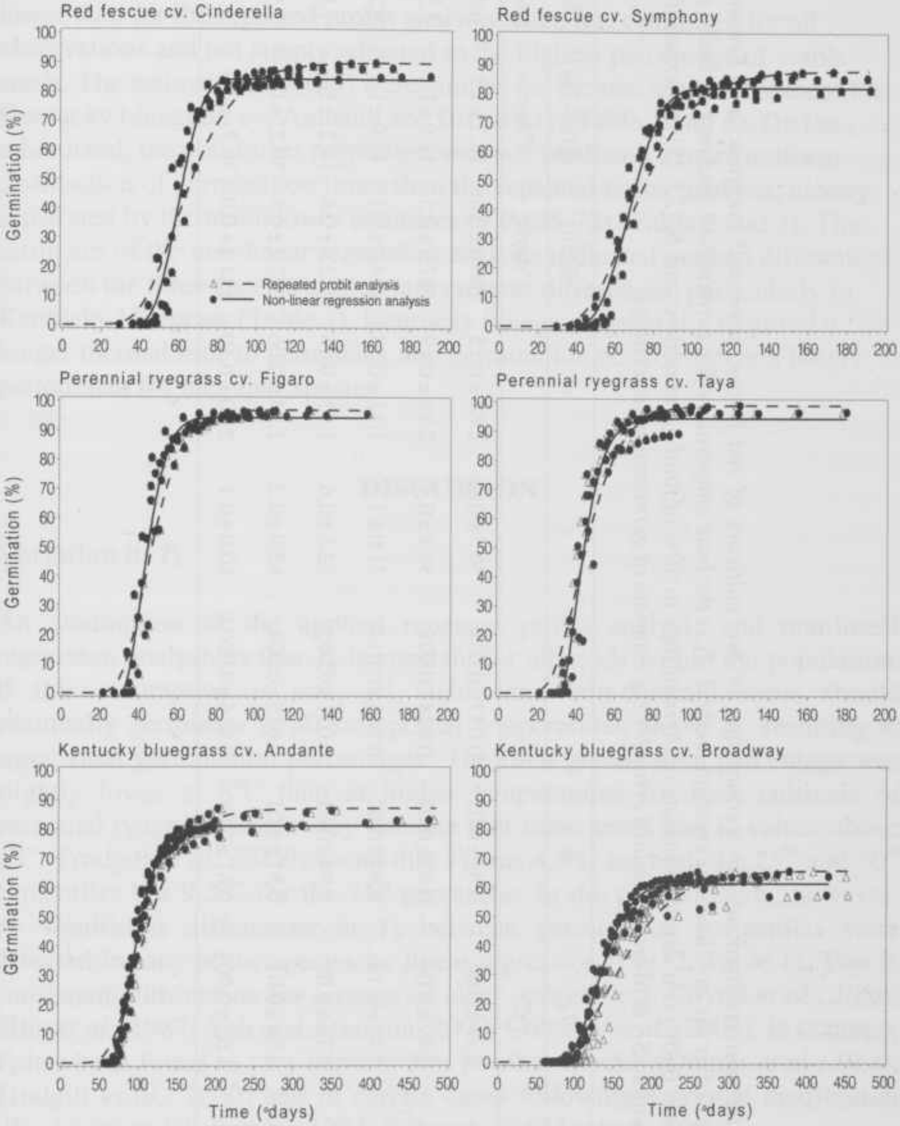

***Figure 3**. Thermal time germination time courses for germination of two cultivars of red fescue, perennial ryegrass, and Kentucky bluegrass, respectively, at five constant temperatures. The time courses were normalized on a thermal time scale with a base temperature estimated by either repeated probit analysis or non-linear regression analysis. Symbols indicate observed germination, and lines indicate the time courses predicted by the repeated probit analysis and the non-linear regression analysis, respectively. Note the different time scale for Kentucky bluegrass cultivars.*

Table 3. *Estimates (±SE) of thermal requirements for germination of two cultivars of red fescue, perennial ryegrass, and Kentucky bluegrass, respectively, determined by the non-linear regression method. T_b is the base temperature for germination, and m, k, z, and c are constants of the Weibull function. $\theta_T(50)$ is a derived estimate of thermal time to 50% of final germination, and θ_T (25-75) is thermal time from 25 to 75% of final germination. SE is overall standard error for the estimate of germination percentage.*

Species	Cultivar	T_b	m	k	z	c	$\theta_T(50)$	$\theta_T(25\text{-}75)$	SE
		(°C)					(°days)	(°days)	(%)
Red fescue	Cinderella	2.71±0.06	0.84±0.01	0.037±0.001	36.3±0.6	2.88±0.25	60.0±0.6	12.7±1.1	6.4
	Symphony	2.49±0.04	0.82±0.01	0.028±0.001	36.8±0.4	2.83±0.19	67.9±0.5	17.0±1.2	5.5
Perennial ryegrass	Figaro	3.43±0.14	0.94±0.02	0.063±0.014	31.0±3.3	1.81±0.53	44.0±1.1	11.0±1.1	8.2
	Taya	3.76±0.09	0.94±0.01	0.071±0.003	32.5±0.6	1.53±0.15	43.6±0.6	11.2 ±1.1	7.8
Kentucky bluegrass	Andante	3.54±0.01	0.81±0.01	0.025±0.001	65.0±0.2	1.64±0.06	97.1±0.5	30.2 ±1.3	3.9
	Broadway	1.65±0.004	0.61±0.01	0.016±0.0003	80.0±0.1	2.10±0.10	134.1±0.9	39.7 ±2.0	3.7

lower than for the repeated probit analysis since it is optimised for all observations and not simply adjusted to the highest percentage of viable seeds. The estimates of $\theta_T(50)$ were similar for the two methods, except for Kentucky bluegrass cv. Andante and Broadway (Table 2 and 3). On the other hand, the non-linear regression analysis predicted a more uniform distribution of germination times than the repeated probit analysis, clearly illustrated by the much lower estimates of $\theta_T(25\text{-}75)$ (Table 2 and 3). The estimates of the non-linear regression analysis indicated marked differences between the three species and certain cultivar differences, particularly in Kentucky bluegrass (Table 3). Kentucky bluegrass generally required a longer thermal time to germinate, and germination occurred over a longer period than for the other species.

DISCUSSION

Variation in T_b

An assumption of the applied repeated probit analysis and non-linear regression analysis is that T_b is constant for all seeds within the population. If this assumption is true, all viable and non-dormant seeds should eventually germinate at all suboptimal temperatures above T_b, resulting in equal final germination percentages. The final germination percentage was slightly lower at 8°C than at higher temperatures for both cultivars of perennial ryegrass, which may indicate that some seeds had T_b values above 8°C. Trudgill *et al.* (2000) found that T_b was 4.8°C for both the 25th and 50th percentiles but 9.2°C for the 75th percentile. In the present study, however, no significant differences in T_b between germination percentiles were detected for any of the species by linear regression (Fig. 2, Table 1). This is consistent with results for a range of other species (e.g. Covell *et al.*, 1986; Ellis *et al.*, 1987; Yeh and Atherton, 2000; Colbach *et al.*, 2002). In contrast, T_b has been found to vary more or less in other species (Dumur *et al.*, 1990; Trudgill *et al.*, 2000) and in certain cases following a normal distribution (Washitani and Takenaka, 1984; Kebreab and Murdoch, 1999).

As pointed out by Ellis *et al.* (1986) and Phelps and Finch-Savage (1997), the method for estimating T_b by linear regression of $1/t_g$ against temperature is problematic since it is a two-step procedure, which does not use the original data in the regression. Despite t_g being estimated reasonably well in step one (Fig. 1), some deviation is inevitably carried over to the second step of the analysis and potentially affecting the regression and hereby reducing the confidence of the estimates of T_b and $\theta_T(g)$. Besides, the method extrapolates beyond the observed temperature range, which may contribute further to the error (Phelps and Finch-Savage, 1997). The method can provide useful indications of the validity of the thermal time model (equation [1] and [2]) such as the linearity of the relationship between temperature and germination rate and of the variation in T_b. Moreover, the method provides a means of detecting optimal temperature for germination, i.e. the temperature providing the maximal germination rate (Garcia-Huidobro *et al.*, 1982). However, for more precise estimation of the thermal requirements at suboptimal temperatures, a one-step analysis should be applied, based on the original data and accounting for whole population behaviour.

Comparison of repeated probit analysis and non-linear regression analysis

The fact that observations from all temperatures converge to a common thermal time germination course (Fig. 3) demonstrates that the assumptions behind the thermal time model (equation [1] and [2]) are fulfilled, viz. that T_b is constant for individual seeds within a population whereas θ_T varies with g. The repeated probit analysis and the non-linear regression analysis both seek to describe the distribution of the thermal time required for germination among seeds within a population, and the latter method proved superior for the present data.

For both population-based methods, variation in thermal time was minimized by optimising T_b which appears to result in similar estimates of T_b, except for Kentucky bluegrass cv. Broadway (Table 2 and 3). For this cultivar, the lower estimate of T_b by the repeated probit analysis was partly due to the germination behaviour at 20ºC where germination was more skewed (Fig. 1) and thus affecting the repeated probit analysis more than the non-linear regression analysis. Consequently, if observations from 20ºC were excluded, T_b estimated by the repeated probit analysis was increased from 0.46ºC to 0.93ºC (R^2=0.87). T_b estimates from the linear regression method (Table 1) are generally higher than those predicted by the population-based models (Table 2 and 3). Since both population-based methods use all observations in the estimation of T_b, and since they also avoid the mentioned problems of two-step analysis and extrapolation beyond the data range, these estimates of T_b must be considered more reliable.

The repeated probit analysis has been used successfully by e.g. Covell *et al.* (1986) and Dahal *et al.* (1990), either using a normal or log-normal distribution of thermal time. Low and high germination percentages, however, may affect the result of the analysis. Thus, Dahal *et al.* (1990)

excluded observations above 90% germination, and both Covell *et al.* (1986) and Steinmaus *et al.* (2000) obtained a better fit when excluding observations below 10% and above 80 or 90% germination. Covell *et al.* (1986) argued that when relating results to field emergence, it is only necessary to consider the range from 10 to 90% cumulative germination. In the present study, the repeated probit analysis also performed considerably better when observations above 90 or 85% were excluded. When including all observations in the analysis, the method predicted thermal time to 50% germination reasonably well but proved unable to sufficiently describe the distribution in thermal time to germination. Consequently, the repeated probit analysis did not provide a sufficiently good fit of the observed data unless a proportion of the observations were excluded, which is unsatisfactory.

In contrast, the non-linear regression analysis provided adequate prediction of both thermal time to 50% germination and the distribution of thermal time to germination (Fig. 3). The better performance of the non-linear regression analysis is primarily due to the ability to account for skewness in time to germination. The *c* parameter of the Weibull function provides a larger flexibility since it can account for skewness (Brown and Mayer, 1988). If *c* is below 3.25, the distribution of time to germination is positively skewed (Brown and Mayer, 1988), and this is the case in all six seed lots in this study (Table 3). Although logarithmic transformation of positively skewed data may result in normal distribution (Scott *et al.*, 1984), the ln-transformation was not sufficient for the present data. The results are consistent with the results of Dumur *et al.* (1990) who also found that the Weibull function was superior in describing cumulative germination curves compared to the cumulative normal distribution and the logistic function, which is very similar to the probit function (Bradford, 1995).

The present data demonstrate that the repeated probit analysis and the non-linear regression analysis generally provide similar and reliable estimates of T_b and $\theta_T(50)$, although the latter method may in certain cases estimate T_b more robustly than the former. However, the distribution of the thermal time to germination was clearly better described by the non-linear regression analysis. Thus, when information about the distribution of the thermal time to germination is sought, and when this distribution is skewed, data should be analysed by the non-linear regression method rather than the repeated probit analysis. Once the parameters of the non-linear regression model (equation [6]) have been estimated for a seed lot, thermal time germination courses as well as real time germination courses at any suboptimal temperature can then be predicted adequately for the whole seed population.

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