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Analysis of flowering dynamics heritability in the perennial warm-season grass *Paspalum dilatatum*

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Abstract

Understanding the flowering cycles of perennial warm-season grass species may be very important to the design of management practices and breeding. However, developmental dynamics are not well understood. As most plant traits associated with flowering dynamics do not follow a normal distribution, the use of general linear models to describe and compare these variables might be misleading. The aims of this study were (i) to find a methodology to compare panicle accumulation curves and (ii) to estimate heritabilities for flowering curve attributes. Panicle counts were recorded during a complete flowering cycle on a diverse collection of dallisgrass (*Paspalum dilatatum*). We compared the efficiency of different linear mixed models based on whole plot or individual plant data; then, we adjusted nonlinear regression curves for individual plants to estimate several curve attributes and compared this approach to the area under the curve. Finally, we calculated the broad-sense heritabilities of the estimated curve parameters. The following reproductive curve attributes were obtained: panicle production potential, panicle accumulation rate and days until 3, 5, 10 and 15 panicles. We found that monitoring individual plants is more efficient when studying flowering attributes. Significant differences among genotypes for several flowering cycle attributes were found. Heritabilities were very high for all flowering cycle initiation and duration attributes. We also showed that the number of days until the emergence of a given low number of panicles can be used as a highly heritable measure to characterize flowering cycles.

Keywords: flowering time, seeds, dallisgrass, harvest date, modelling

Introduction

Warm-season grasses are a key component of temperate grasslands. Genetic improvement of grasses through breeding has led to considerable progress by increasing feeding value, extending the grazing season and improving the persistence of pastures (Wilkins and Humphreys, 2003). However, breeding programmes leading to the domestication of perennial warm-season grasses have been less successful than those of temperate grasses. Some of the main challenges in breeding warm-season grasses are managing their growth cycle, flowering asynchrony and harvesting seeds (Miles, 2001). Most perennial warm-season grasses have indeterminate flowering dynamics, and consequently, a given plant may bear vegetative and reproductive tillers at the same time (Moore and Moser, 1995). Such absence of a clear differentiation between the vegetative and reproductive phases in perennial grasses adds great complexity to forage production and animal grazing management (Mitchell *et al.*, 1997). The underlying processes of tillering, flowering and bud-development dynamics of perennial warm-season grasses are poorly understood (Ott and Hartnett, 2011; Williamson *et al.*, 2012). In annual species, these processes are well characterized (Mace *et al.*, 2013); however, the complexity of perennial grasses in terms of their morphological development leads to a lack of consensus on methods for describing and quantifying developmental stages (Moore and Moser, 1995). Because genetic gains could be obtained through selection of developmental traits, it is relevant not only to characterize the morphological develop-

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ment of the plant but also the estimation of the heritability of its attributes which has practical implications for breeding (Van Esbroeck *et al.*, 1998).

Flowering time and dynamics are important, and complex traits and the strategies to best describe them have varied considerably. Several approaches have been proposed to characterize flowering dynamics such as the change over time in panicle number per square metre (Mitchell *et al.*, 1997), days until the emergence of the first panicle (Moore and Moser, 1995) and mean inflorescence stage in species with multiple flowering tillers (Moore *et al.*, 1991). Appropriate statistical models are needed in this context to describe and compare these variables, because most of them do not follow a normal distribution, and therefore, the use of general linear models as a statistical approach may be inappropriate. To solve this problem, methods to linearize models such as data transformation have been proposed. The use of linear mixed models on the transformed data allowed working with this type of distribution while modelling correlations in time (Molenberghs and Verbeke, 2007). An alternative is the use of generalized linear mixed models (GLMM) (Breslow and Clayton, 1993) which are obtained from generalized linear models that incorporate random effects within linear predictors. These models are useful when the objective is modelling the response of dependent variables in longitudinal studies to assess variables with overdispersion in binomial or Poisson distributions (Pan and Lin, 2005). Another approach, such as the analysis of the area under the curve (AUC), has been applied in many contexts as a tool to study the evolution of normal and non-normal data such as counts (Lobo *et al.*, 2008). This approach has some limitations as different formulas are used to derive the AUC, and its interpretation in repeated measurements over time is not straightforward (Pruesner *et al.*, 2003). Nonlinear models are often used to analyse the evolution of a variable in studies of physiological responses in plants, and they are preferred to polynomial models because the coefficients obtained in curve fitting are biologically meaningful (Peek *et al.*, 2002). Yet another alternative for analysing the evolution of a variable along time is the adjustment of nonlinear regression models, where information on potential production and the evolution over time of each curve can be obtained. Classical model approaches that ignore the correlations that may exist result in two problems: inefficient estimates of regression parameters and inconsistent precision estimates (Zeger and Liang, 1992). Therefore, it is desirable to evaluate statistical approaches that model the covariance matrix of measurements repeated in time over the same experimental subjects in terms of their efficiency. The goal of this study was to evaluate the performance of different modern statistical tools to describe the dynamics of panicle emergence in a perennial grass.

Dallisgrass (*Paspalum dilatatum* Poir.) is a perennial warm-season grass widely recognized for its productive potential (Pizarro, 2002). Significant levels of natural genetic variability in the species have been assembled in available collections that are currently being evaluated to differentiate flowering curves (Speranza, 2005). Because all biotypes are highly apomictic or selfing perennials, repetitions of the same genetic material can be easily obtained and broad-sense heritabilities estimated. To begin to understand how to characterize these traits for selection or management purposes, we analysed the complete flowering curves of a diverse collection during one flowering season. To perform this analysis, we recorded panicle appearance over time on a diverse collection of *P. dilatatum* genotypes and tested different statistical strategies such as the adjustment of regression curves and the incorporation of repeated measures in time, to compare flowering curves and estimate the broad-sense heritability of different attributes of the flowering curves.

Material and methods

Plant material and data collection

Twelve pentaploid genotypes of *P. dilatatum* that are considered to represent the natural genetic diversity were used. The sampling was based on Speranza (2009) and further unpublished data. Plant material was obtained from the Germplasm Bank of the Facultad de Agronomía (Universidad de la República, Uruguay). Twelve pentaploid clones from four genetic groups (group A, B, C, D) were used. Individual plants from each genotype were grown in pots in a greenhouse during 1 year. Seedlings were transplanted to the experimental field in the autumn of 2011. The trial was located in Sayago, Montevideo, Uruguay. The soil of the experimental field was a typic argiudoll, with 3·3% organic matter and 18 ppm phosphorus. No particular deviation from historical rainfall and mean temperatures was recorded prior to or during the experiment (Table 1).

The experimental design was a completely randomized design with three replicates. Each plot consisted of six plants arranged in two rows with a separation of 15 cm between individuals. The distance among plots was 30 cm. The number of emerged panicles was recorded in spring 2011 on each individual plant every 3–4 days between November and December. A panicle was considered emerged when the whole inflorescence was visible.

Table 1 Average monthly temperature, humidity, accumulated rain and accumulated evaporation between October and December of 2011 at the experimental site.

Month	Temperature			Average relative humidity (%)	Accumulated rain (mm)	Accumulated evaporation (mm)
	Máximum (°C)	Mínimum (°C)	Mean (°C)			
October	19·2	11·6	15·4	74·2	51·5	169·3
November	25·4	14·5	20·0	61·7	110·6	245·6
December	24·4	15·6	20·0	68·0	78·6	257·1

Statistical analysis

Individual plant assessment

All the data collected were analysed using Statistical Analysis Software Procedures of SAS (SAS Institute Inc, 2005) and Infostat Software (Di Rienzo *et al.*, 2012). Individual plant information was used to determine whether using data from individual plants over time was relevant. We evaluated four statistical models involving different combinations of correlation structures (i.e. compound symmetry or autoregressive) and units of study (i.e. plot or individual plant): (i) whole-plot average data with a compound symmetry correlation in time for each plot; (ii) whole-plot average data with an autoregressive correlation (AR1) in time for each plot; (iii) single plant data with a compound symmetry correlation in time for each individual plant, and (iv) single plant data with an autoregressive (AR1) correlation in time for each individual plant. Model selection was based on likelihood ratio tests.

Flowering characterization

To explore the relationships among modelled panicle accumulation dynamics for each genotype, a logistic regression model was fitted for each plant:

$$Y_{ij} = \frac{b_0}{1 + b_1 e^{-b_2 x_i}}$$

where Y_{ij} is the panicle number accumulated until the i-th day for the j-th genotype, b_0, b_1 and b_2 are curve parameters and x_i is the number of days from the start of the experiment. The regression models were fitted in SAS (SAS Institute Inc., 2005) using PROC NLIN.

The following attributes of the regression curve were used to characterize genotypes:

1 Potential number of panicles (b_0).
2 Panicle accumulation rate ($b_1 e^{-b2}$).
3 Number of days to 3, 5, 10 and 15 panicles.
4 Number of days to 50% and 80% of the potential number of panicles.
5 Area under the curve (AUC)

The theoretical potential number of panicles was calculated from the b_0 term of the adjusted logistic regressions, and the panicle accumulation rate was calculated as a product of the $b_1 * e^{(-b2)}$ relationship between parameters. The number of days to the emergence of the first 3, 5, 10 and 15 panicles, and the number of days to 50% and 80% of b_0 were obtained using the adjusted parameters for each curve. The AUC was calculated by integrating over the fitted logistic regression model for each plant through the 'areaxy' function of Infostat Statistical Software (Di Rienzo *et al.*, 2012). Each one of the attributes of the panicle accumulation curve was analysed with the following linear mixed model:

$$Y_{ijk} = \mu + \beta_i + G_j + \varepsilon_{ij} + \delta_{ijk}$$

where Y_{ijk} is the response variable of interest, μ is the overall mean, β_i is the i-th replication, G_j is the j-th genotype, ε_{ij} is the experimental error and δ_{ijk} is the sub sampling or plant error. Adjusted means for each attribute for each genotype were estimated, and the Tukey's test was used when the ANOVA detected significant differences among genotypes ($\alpha = 0{\cdot}05$).

To determine the prediction power of the adjusted curves, we obtained the coefficient of determination (R^2) as an indication of the model fit for each individual plant as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}{\sum_{i=1}^{n}(Y_i - \bar{Y}_i)^2}$$

where R^2 is the coefficient of determination; y_i are the observed panicle count values for each individual plant, $\hat{Y}_i$ is the predicted value for that model and $\bar{Y}$ its mean value.

Variance components and heritability

Variance components for days to emergence of the first 3, 5, 10 and 15 panicles, days to the inflection point, panicle accumulation rate and theoretical

potential number of panicles were estimated with PROC MIXED of SAS using the model described above but including genotype as a random effect. With these variance component estimations, broad-sense heritabilities on a mean basis were estimated for each of the attributes of the panicle cumulative curve:

$$H^2 = \frac{V_g}{V_g + (V_e/r)}$$

where H^2 is the heritability, V_g is the genotypic variance, V_e is the residual variance and r is the number of replicates. Additionally, in order to describe the association among curve attributes related to the onset of the reproductive stage, Pearson's correlation coefficients among traits and the ranking of the genotypes for these traits were compared.

Results

Model comparison

Models based on individual plant data had a better fit than whole-plot average models (Table 2). The comparison of models that assume a constant correlation among data over time with models that use different structures in the covariance matrix showed that the autoregressive adjustment (AR1) performed significantly better than the compound symmetry model. The autoregressive covariance structure assumes that observations which are more proximate are more correlated than measures that are more distant.

Logistic curve parameters

Statistically significant differences among genotypes were found for some curve attributes (Table 3). No statistical differences were found for potential number of panicles (b_0) and panicle accumulation rate. Potential number of panicles ranged from 32 panicles for genotype B2 to 52 panicles for genotype D2 and for panicle accumulation rate the values ranged from 687 for genotype B3 to 12759 for genotype D3. The number of days to 50% and 80% of b_0 ranged from a minimum of 19 and 23 days for genotype C1 to a maximum of 27 and 31 days for genotype A2 respectively. The number of days to 50% of b_0 was significantly higher in genotypes D1 and A2 compared to genotypes B2 and C1. For number of days to 80% of b_0, genotypes C2 and A2 showed the longest cycles, while the parameter was significantly lower for C1. The AUC was different for the different genotypes (Table 3). Genotypes D2 and B1 had the highest cumulative area, and genotypes C1, C2 and B2 showed the lowest values of AUC. The model fit was very good in most of the cases with R^2 values higher than 0·8 (Table 3).

Heritabilities

Very high broad-sense heritability values were obtained for attributes of the panicle accumulation curves associated with cycle duration or initiation traits ($H^2 > 0{\cdot}80$), including number of days to 50% and 80% of b_0 and days to a given low number of panicles. On the other hand, lower heritabilities were found for potential number of panicles and panicle accumulation rate (Table 4).

Association among flowering date curve attributes

In spite of their nonlinear relationship, the curve attributes that describe the flowering date of a given material produced a very similar ranking of the genotypes as panicle accumulation progresses. However, the order of the genotypes changes for relationships of the curve parameters that include fractions of the potential number of panicles (Fig. 1a). There is a strong correlation among the days until the emergence of 3, 5, 10 and 15 panicles, with the correlation decreasing with the number of panicles. Correlation between days until the emergence of panicles and the number of days to 50 or 80% of the potential number of panicles is low (Fig. 1b).

Table 2 Model comparison for count data where models of whole-plot average with no correlation in time, whole-plot average with autoregressive correlation in time, single plant with compound symmetry correlation and single plant with autoregressive correlation in time were compared by likelihood ratio test (LR test).

Model	2 Res Log Pseudo-Likelihood	LR test
Whole-plot average data with compound symmetry correlation in time	14453·6	
Whole-plot average data with autoregressive correlation in time	9846·0	*
Single plant with compound symmetry correlation in time	7693·6	*
Single plant with autoregressive correlation in time	3539·1	*

*Significant at the probability level of $P < 0{\cdot}05$.

Table 3 Characterization of panicle production curves according to genotype. Adjusted means for potential number of panicles and panicle accumulation rate parameters and comparison of days accumulated at 3, 5, 10 and 15 panicles and number of days to 50% and 80% of b_0.

	Curve attributes		Duration cycle (days to)							
Genotype	Potential number of panicles†	Panicle accumulation rate†	Number of days to 3 panicles	Number of days to 5 panicles	Number of days to 10 panicles	Number of days to 15 panicles	Number of days to 50% of b_0	Number of days to 80% of b_0	Area under the curve (AUC)	Model fit R^2 (s.e.)
A1	50·17	7485	$17{\cdot}77^{a}$	$19{\cdot}24^{a}$	$21{\cdot}41^{a}$	$22{\cdot}89^{ab}$	$24{\cdot}77^{abcd}$	$27{\cdot}95^{abc}$	$501{\cdot}0^{abc}$	0·895 (0·113)
A2	46·81	3943	$16{\cdot}71^{ab}$	$18{\cdot}47^{abc}$	$21{\cdot}10^{abc}$	$22{\cdot}95^{ab}$	$26{\cdot}76^{a}$	$30{\cdot}77^{a}$	$447{\cdot}9^{abcd}$	0·857 (0·172)
A3	49·11	5765	$16{\cdot}62^{ab}$	$18{\cdot}37^{abc}$	$20{\cdot}99^{abc}$	$22{\cdot}85^{ab}$	$25{\cdot}96^{ab}$	$30{\cdot}59^{ab}$	$570{\cdot}7^{ab}$	0·858 (0·157)
B1	46·56	3740	$12{\cdot}88^{bcd}$	$14{\cdot}44^{cde}$	$16{\cdot}75^{de}$	$18{\cdot}32^{cde}$	$21{\cdot}15^{abcd}$	$24{\cdot}64^{abc}$	$631{\cdot}5^{a}$	0·857 (0·145)
B2	31·97	2007	$11{\cdot}68^{cd}$	$13{\cdot}02^{e}$	$15{\cdot}03^{e}$	$16{\cdot}43^{e}$	$19{\cdot}13^{cd}$	$23{\cdot}33^{bc}$	$233{\cdot}3^{d}$	0·883 (0·086)
B3	37·06	687	$11{\cdot}59^{d}$	$13{\cdot}38^{e}$	$16{\cdot}23^{de}$	$18{\cdot}48^{bcde}$	$20{\cdot}04^{bcd}$	$24{\cdot}65^{abc}$	$348{\cdot}0^{cd}$	0·889 (0·071)
C1	37·40	2889	$12{\cdot}16^{cd}$	$13{\cdot}53^{de}$	$15{\cdot}66^{e}$	$18{\cdot}89^{de}$	$18{\cdot}63^{d}$	$22{\cdot}69^{c}$	$249{\cdot}7^{d}$	0·871 (0·079)
C2	36·35	4233	$13{\cdot}28^{bcd}$	$14{\cdot}75^{bcde}$	$17{\cdot}16^{cde}$	$19{\cdot}85^{abcd}$	$25{\cdot}30^{abc}$	$31{\cdot}51^{a}$	$236{\cdot}6^{d}$	0·880 (0·110)
C3	47·23	1867	$17{\cdot}09^{ab}$	$18{\cdot}86^{ab}$	$21{\cdot}53^{a}$	$23{\cdot}44^{a}$	$21{\cdot}26^{abcd}$	$24{\cdot}42^{abc}$	$300{\cdot}1^{cd}$	0·919 (0·097)
D1	49·21	6099	$16{\cdot}19^{abc}$	$17{\cdot}69^{abcd}$	$19{\cdot}93^{abcd}$	$21{\cdot}37^{abcd}$	$26{\cdot}18^{ab}$	$30{\cdot}04^{abc}$	$377{\cdot}6^{bcd}$	0·931 (0·061)
D2	52·03	8706	$15{\cdot}39^{abcd}$	$16{\cdot}93^{abcde}$	$19{\cdot}19^{abcde}$	$20{\cdot}71^{abcde}$	$22{\cdot}78^{abcd}$	$25{\cdot}80^{abc}$	$630{\cdot}2^{a}$	0·840 (0·167)
D3	44·41	12759	$13{\cdot}98^{abcd}$	$15{\cdot}26^{abcde}$	$17{\cdot}20^{bcde}$	$18{\cdot}59^{bcde}$	$21{\cdot}46^{abcd}$	$26{\cdot}40^{abc}$	$306{\cdot}1^{cd}$	0·869 (0·098)

Overall means followed by the same letter are not significantly different at $P < 0{\cdot}05$ according to Tukey. †Non-significant statistical differences.

Table 4 Estimation of variance components (kg ha^{-1})^2 and broad-sense heritabilities (%) for main characteristics of panicle-production curves.

	Variance component			
Trait	**Genotypic (among genotypes)**	**Residual error (among plots)**	**Subsampling error (among plants, within plots)**	**Heritability**
Flowering time				
Days to 3 panicles	4·378	0·744	8·658	0·8955
Days to 5 panicles	4·788	0·539	8·409	0·9061
Days to 10 panicles	5·219	0·408	9·091	0·9068
Days to 15 panicles	5·394	0·327	11·704	0·8865
Number of days to 50% of b_0	10·552	1·650	9·811	0·9480
Number of days to 80% of b_0	7·818	2·594	27·87	0·8330
Curve characteristic				
Potential number of panicles (b_0)	22·077	28·943	213·310	0·6368
Panicle accumulation rate (b_1e^{-b2xi})	5·5487	0·9145	73·5878	0·5002

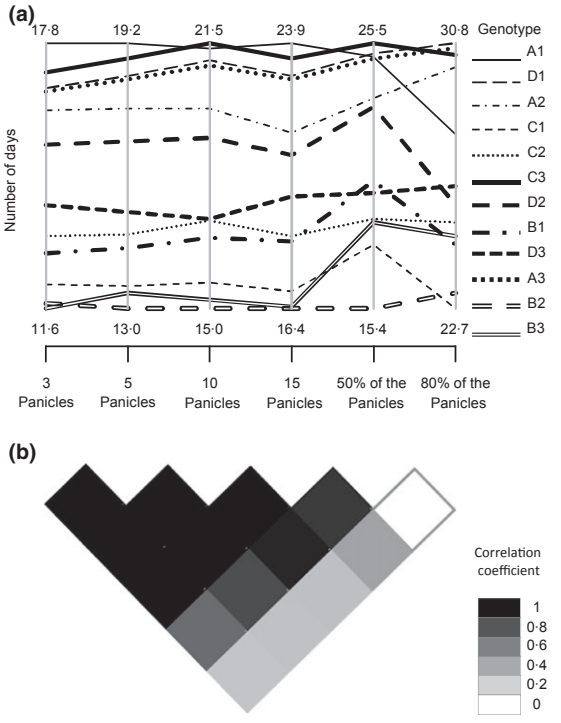

Figure 1 Association among curve attributes on the modelled curves. (a) Ranking of the adjusted genotypic means for days to 3, 5, 10 and 15 emerged panicles and number of days to 50% and 80% of the potential number of panicles per plant. (b) Linear correlations among the curve attributes.

Discussion

There is no general agreement on the actual physiological and developmental processes that explain variations in flowering date and panicle number, although temperature has been reported as the primary factor that defines the time between tiller and inflorescence emergence (Pearson and Shah, 1981). Several studies have described morphological development in response to environmental variation such as photoperiod and cumulative degree days in relation to biomass production and quality (Sanderson and Wolf, 1995; Mitchell *et al.*, 1997). In perennial warm-season grasses, stem developmental stages within a given plant can vary widely (Moore *et al.*, 1991). There are results that show that variations in time to flowering are directly associated with variations in leaf appearance rates in switchgrass (Van Esbroeck *et al.*, 1998) or variation in total number of leaves to flowering in switchgrass (Van Esbroeck *et al.*, 1998) and maize (Van Esbroeck *et al.*, 2008). The use of accurate models to describe the progression of flowering of individual plants is essential to improve the statistical analysis of this complex process as well as to estimate model parameters of likely physiological meaning.

Analysis models

Our results show that models that utilize information from individual plants had better fits compared to those that only use average plot information. Additionally, comparing different strategies to model the variance–covariance matrix, we found that the autoregressive structure had the best fit. These results are consistent with other studies on seed production and flowering attributes in grasses, where different variance–covariance estimation approaches were compared (Venuto *et al.*, 2002; Sahramaa *et al.*, 2004). The autoregressive adjustment is a structure where the count variable depends on its own previous values

(Little *et al.*, 2006); thus, it is advantageous to follow each plant individually and incorporate this information into the model of analysis if possible. Furthermore, as the flowering process is asynchronous among individual tillers, mean values from several individuals may lead to an inaccurate description of flowering. Sampling protocols, as well as data analysis and interpretation can be further enhanced by taking into account the differential response of different tiller age classes. This would enable the establishment of meaningful relationships between flowering dynamics and environmental conditions.

Our experimental data were well described by the logistic regression models, and the adjusted curves were successfully used to extract several individual attributes of flowering behaviour, allowing the comparison of genotypes in terms of potential panicle accumulation, panicle accumulation rate and days to the emergence of 3, 5, 10 and 15 panicles. From a practical point of view, these measurements can be informatively used for germplasm characterization in perennial warm-season grasses with indeterminate flowering. Differences among genotypes for days to the emergence of a given low number of panicles were statistically significant. Despite the underlying nonlinear association among these variables, the ranking of the genotypes resulting from all of them remained generally the same. On the other hand, the ranking of genotypes for days until 50 or 80% of the potential number of panicles is different, probably due to the different potential number of panicles of the genotypes. Therefore, correlation among days to the number of 3, 5, 10 and 15 panicles remains high enough to justify the use of few panicles when characterizing flowering dates in big germplasm collections.

The AUC analysis as a statistical tool has successfully been used in areas such as medicine and life sciences in general. However, this methodology is not appropriate for all cases (Austin, 2007), and its predictive abilities were reported to be limited because of problems of parameter overestimation (Manel *et al.*, 2002). Because of its widespread use, evaluation of this methodology was considered necessary in this particular context. In our case, differences between genotype means were found using the AUC; however, it showed several limitations for the description of panicle-emergence dynamics. The same AUC value can be explained by different combinations of curve attributes. For example, when comparing genotypes A2 and C1, both materials had non-significantly different low AUC values; however, the number of days to 3, 5, 10 and 15 panicles, and the number of days to 50% and 80% of the potential number of panicles were significantly different (Fig. 2). For this reason, parameter characterization of each curve was considerably more informative than the AUC analysis.

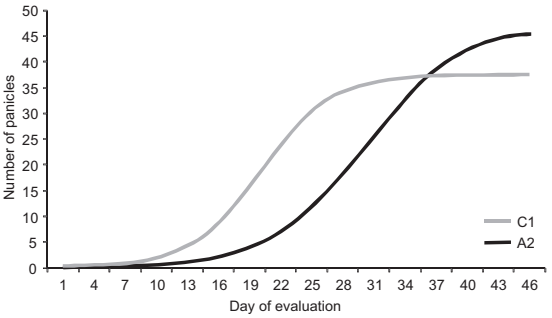

Figure 2 Comparison of the adjusted curves for two genotypes with AUC that did not differ significantly in the experiment.

Variability and heritability

Statistical analysis of panicle accumulation curves showed a different behaviour in flowering time when analysed by genotype. The estimation of number of days to 50% of the potential number of panicles is informative because it is the point of maximum panicle accumulation rate. It has been shown that close to 10 days after panicle emergence, *Paspalum* seeds ripen and detach from the mother plant (Burson *et al.*, 1978). Consequently, after the same period of time from the number of days to 50% of the potential number of panicles, the highest number of seeds available for harvest is expected to occur. The number of days until the maximum rate in the panicle accumulation curve showed differences among genotypes that exceeded 1 week in several cases which were not statistically significant. Likewise, no statistically significant differences were found among genotypes for potential number of panicles. Because of the implications these parameters may have for management, it is probably reasonable to further analyse these differences using more powerful experimental designs and multiple environments.

The analysis of environmental influence on the reproductive behaviour of these genotypes was approached through the estimation of the heritabilities of the cumulative curve attributes during a flowering season. High broad-sense heritability estimates have been found for days to flowering in other grasses; for example, heritability values higher than 90% have been reported both for summer annual grasses such as rice (Sadegui, 2011; Seyoum *et al.*, 2012) and perennials such as switchgrass (Van Esbroeck *et al.*, 1998). The high heritability calculated for days to a given

number of panicles suggests that differences among genotypes in a given environment for the onset of flowering in each growing season are largely controlled by the genetic regulation of some morphophysiological trait. In other warm-season perennial grass species, the number of phytomers per tiller is considered to determine the time elapsed between tiller emergence and anthesis, and it can be interpreted as an indicator of adaptation to the length of the growing season in its region of origin (Casler *et al.*, 2004). Compared to other morphological tiller traits, the number of phytomers per tiller shows lower environmental plasticity (Boe and Casler, 2005). On an individual plant basis, it is expected that the final number of panicles is affected by the potential number of tillers initiating growth in a given season (Ott and Hartnett, 2012). Potential number of panicles and panicle accumulation rate are known to be strongly influenced by environmental conditions (Ott and Hartnett, 2011), in agreement with our own results for those traits (Table 4).

Conclusions

This study successfully characterized genetic variability in panicle-production curves within a diverse collection of pentaploid *P. dilatatum* genotypes during one season. The approach can be applied in the future to different collections or environmental conditions to further characterize the process and its biological basis. We showed by using individual plant information and modelling, that the correlation of repeated measurements helped to effectively describe panicle accumulation curve attributes by the use of nonlinear regression models. Curve attributes related to the timing of the onset of flowering were highly heritable as was the moment of highest rate of panicle emergence. Consequently, selection may be applied to this collection for these traits. From a practical point of view, the number of days to the emergence of the first 3, 5, 10 or 15 panicles is an easily recorded heritable trait that can be used instead of a full characterization of reproductive behaviour to evaluate the phenological variability of a collection. We have also shown that regardless of the nonlinear relationship among the curve attributes, the number of days to the emergence of the first few panicles yields a genotypic ranking that is the same as the ranking when more panicles are considered. Our study focused on designing an approach to effectively describe the observed variability in flowering dynamics in a variable germplasm collection. To gain further understanding of the physiological basis of each component, a thorough description of tiller developmental morphology and the possible interactions of the estimated genetic components with environmental factors such as photoperiod and/or temperature should be analysed.

References

Austin M. (2007) Species distribution models and ecological theory: a critical assessment and some possible new approaches. *Ecological Modelling*, **200**, 1–19.

Boe A. and Casler M. (2005) Hierarchical analysis of switchgrass morphology. *Crop Science*, **45**, 2465–2472.

Breslow N. and Clayton D. (1993) Approximate inference in generalized linear mixed models. *Journal of the American Statistical Association*, **88**, 9–25.

Burson J., Correa J. and Potts H. (1978) Anatomical study of seed shattering in bahiagrass and dallisgrass. *Crop Science*, **18**, 122–123.

Casler M.D., Vogel V.P., Taliaferro C.M. and Wynia R.L. (2004) Latitudinal adaptation of switchgrass populations. *Crop Science*, **44**, 293–303.

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M. and Robledo C.W. (2012) *InfoStat versión 2012*. Argentina: Grupo InfoStat, FCA, Universidad Nacional de Córdoba. URL http://www.infostat.com.ar

Little R.C., Milliken G.A., Stroup W.W., Wolfinger R.D. and Schabenberger O. (2006) *SAS for mixed models*. Cary, NC: SAS Institute Inc.

Lobo J.M., Jimenez-Valverde A. and Real R. (2008) AUC: a misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography*, **17**, 145–151.

Mace E.S., Hunt C.H. and Jordan D.R. (2013) Supermodels: sorghum and maize provide mutual insight into the genetics of flowering time. *Theoretical and Applied Genetics*, **126**, 1377–1395.

Manel S., Williams H.C. and Ormerod S.J. (2002) Evaluating presence-absence models in ecology: the need to account for prevalence. *Journal of Applied Ecology*, **38**, 921–931.

Miles J.W. (2001) Achievements and perspectives in the breeding of tropical grasses and legumes. Proceedings of the XIX International Grassland Congress, pp. 509–515. Sao Paulo, Brazil.

Mitchell R.B., Moore K.J., Moser L.E., Fritz J.O., Redfearn D.D., Rob B. and Fritz J. (1997) Predicting developmental morphology in switchgrass and big bluestem. *Agronomy Journal*, **89**, 827–832.

Molenberghs G. and Verbeke G. (2007) *Models for discrete longitudinal data*. New York: Springer Verlag.

Moore K.J. and Moser L.E. (1995) Quantifying developmental morphology of perennial grasses. *Crop Science*, **35**, 37–43.

Moore K.J., Moser L.E., Vogel K.P., Waller S.S., Johnson B.E. and Pedersen J.F. (1991) Describing and quantifying growth stages of perennial forage grasses. *Agronomy Journal*, **83**, 1073–1077.

Ott J.P. and Hartnett D.C. (2011) Bud production and dynamics of flowering and vegetative tillers in

Andropogon gerardii (Poaceae): the role of developmental constraints. *American Journal of Botany*, **98**, 1293–1298.

Ott J.P. and Hartnett D.C. (2012) Contrasting bud bank dynamics of two co-occurring grasses in tallgrass prairie: implications for grassland dynamics. *Plant Ecology*, **213**, 1437–1448.

Pan Z. and Lin D.Y. (2005) Goodness-of-fit methods for generalized linear mixed models. *Biometrics*, **61**, 1000–1009.

Pearson C.J. and Shah S.G. (1981) Effects of temperature on seed production, seed quality and growth of *Paspalum dilatatum*. *Journal of Applied Ecology*, **18**, 897–905.

Peek M., Russek-Cohen E., Wait A. and Forseth I. (2002) Physiological response curve analysis using nonlinear mixed models. *Oecologia*, **132**, 175–180.

Pizarro E. (2002) Forage potential of the *Paspalum* genus. Tropical Pastures, **21**, 38–46.

Pruesner J.C., Kirschbaum C., Meinlschmid G. and Hellhammer D.H. (2003) Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*, **28**, 916–931.

Sadegui S. (2011) Heritability, phenotypic correlation and path coefficient studies for some agronomic characters in Landrace rice varieties. *World Applied Sciences Journal*, **13**, 1229–1233.

Sahramaa M., Hommo L. and Jauhiainen L. (2004) Variation in seed production traits of reed Canarygrass germplasm. *Crop Science*, **44**, 988–996.

Sanderson M.A. and Wolf D.D. (1995) Morphological development of switchgrass in diverse environments. *Agronomy Journal*, **87**, 908–915.

SAS Institute Inc. (2005). *SAS User's guide: Statistics*. Cary, NC, USA: SAS Institute Inc.

Seyoum M., Alamerew S. and Bountte K. (2012) Genetic variability, heritability, correlation coefficient and path analysis for yield and yield related traits in upland rice (*Oryza sativa*, L.). *Journal of Plant Science*, **7**, 13–22.

Speranza P. (2005) The challenges of the exploration of genetic resources in apomictic plants: lessons from *Paspalum dilatatum*. *Agrociencia (Uruguay)*, **9**, 73–76.

Speranza P. (2009) Evolutionary patterns in the Dilatata group (*Paspalum*, Poaceae). *Plant Systematics and Evolution*, **282**, 43–56.

Van Esbroeck G.A., Hussey M.A. and Sanderson M.A. (1998) Selection response and developmental basis for early and late panicle emergence in Alamo switchgrass. *Crop Science*, **38**, 342–346.

Van Esbroeck G.A., Ruiz Corral J.A., Sanchez Gonzalez J.J. and Holland J.B. (2008) A comparison of appearance rates among teosinte, maize landraces and modern maize. *Maydica*, **53**, 117–123.

Venuto B.C., Redfearn D.D., Pitman W.D. and Alison M.W. (2002) Seed variation among annual ryegrass cultivars in south-eastern USA and the relationship with seedling vigour and forage production. *Grass and Forage Science*, **57**, 305–311.

Wilkins P.W. and Humphreys M.O. (2003) Progress in breeding perennial forage grasses for temperate agriculture. *Journal of Agricultural Science*, **140**, 129–150.

Williamson M.M., Wilson G.W. and Hartnett D.C. (2012) Controls on bud activation and tiller initiation in C 3 and C 4 tallgrass prairie grasses: the role of light and nitrogen. *Botany-Botanique*, **90**, 1221–1228.

Zeger S. and Liang K. (1992) An overview of methods for the analysis of longitudinal data. *Statistics in Medicine*, **11**, 1825–1839.