ReseaRch

Phenotypic recurrent selection has been the most commonly used procedure by breeders of perennial forages (Vogel and Pedersen, 1993; Wilkins and Humphreys, 2003; Conaghan and Casler, 2011). The purpose of recurrent selection is to continuously increase the frequency of favorable alleles for traits under selection in the breeding population. In contrast, genotypic selection involves selection of desirable parent genotypes followed by progeny tests to evaluate breeding value performance of the parents and cultivar construction from a limited number of parents (Casler and Brummer, 2008). The experimental evaluation and selection methods can vary according to the type of progeny and estimation method. Progenies may consist of half-sibs or full-sibs, and selection pressure can be applied among families, within families, or both.

The breeder’s decision on the best selection method is conditioned by the following factors: target species, mating system, available germplasm, target population of environments for the new potential cultivar, field-evaluation requirements for the species of interest, the ability to place meaningful selection pressure...
on individual progeny within families, and the type of cultivar to be developed (synthetic, vegetatively propagated genotype, or hybrid). Breeders are also concerned with rate of gain or selection efficiency, necessitating an evaluation of both cycle time and selection accuracy.

Breeding systems to improve cross-pollinated perennial grasses have been designed using phenotypic selection with numerous modifications and options (Vogel and Pedersen, 1993), including phenotypic individual selection within half-sib families (Ravel and Charmet, 1996; Casler, 2010) and various among-and-within-half-sib family selection methods (Oliveira, 1992; Casler, 2008; Casler and Brummer, 2008). Generalized linear mixed model approaches involving the use of best linear unbiased prediction (BLUP) methods to estimate genotype breeding values are far less commonly used in forage breeding (Resende et al., 2004, 2006, 2007; Figueiredo et al., 2012). Formal comparisons of these BLUP selection procedures with more traditional selection methods are lacking in the forage breeding literature.

Due to difficulty in placing meaningful selection pressure on genotypes within families, family selection methods have been considered highly inefficient for many years (Breese and Hayward, 1972). Casler (2008) discussed the need to capture within-family genetic variability, particularly for complex traits such as forage or biomass yield. Selection of favorable genotypes within families can result in significant gains for forage or biomass yield, even in situations where within-family selection was based on indirect selection for a surrogate trait (Casler, 2008, 2010). Use of the full spectrum of additive genetic variance is critical due to the lack of historical gains for forage yield and the need to increase biomass yield in bioenergy production systems (Casler and Brummer, 2008; Bhandari et al., 2010).

Low predictive ability of commercial productivity when selection is based on individual plants limits advances in forage breeding, mostly because lack of intergenotypic competition in spaced-plant nurseries severely limits their value for improvement of complex traits such as forage or biomass yield (Wilkins and Humphreys, 2003; Missaoui et al., 2005; Casler and Brummer, 2008). The genetic correlation between spaced-plant and sward-plot estimates of forage yield is highly variable and unpredictable, for example, \( r = 0.66 \) in alfalfa (\( Medicago sativa \) L.) (Annichiarico, 2006), \( r = 0.60 \) in white clover (\( Trifolium repens \) L.) (Annichiarico and Piano, 2000), \( r = 0.37 \) in tall fescue (\( Festuca arundinacea \) Schreb.) (Waldron et al., 2008), and \( r = 0.00 \) in smooth bromegrass (\( Bromus inermis \) Leyss.) (Carpenter and Casler, 1990). Due to the effects of intergenotypic and intraspecific competition in forage breeding, complex traits such as forage or biomass yield should be considered as different traits in spaced-plant vs. sward-plot settings, such that correlated responses to selection become part of the expected gain equations (Real et al., 2000; Annichiarico, 2006).

Predicting performance under conditions of intergenotypic competition requires specific and uniformly applied plot methods (Vogel and Pedersen, 1993), a problem that is not unique to forage breeding but common to annual grain crops (Jearakongman et al., 2003; Hansen et al., 2005), forest trees (Resende et al., 2005), and sugarcane (\( Saccharum officinarum \) L.) (Milligan et al., 2007). There is a prolific literature regarding methods to achieve uniform intergenotypic competition in selection programs, including choice of plot size, seeding rates, alternative competitor species, and statistical methods (e.g., England, 1977; Casler and Brummer, 2008; Stringer et al., 2011). Due to variation in breeding objectives, species biology, environmental factors, and breeder’s philosophies, there is little consensus about the best approaches to solve this problem.

Based on the considerations above, exemplifying a wide range in potential breeding methods for forage and biomass crops, our goal was to develop models to (i) compare various selection methods commonly used in perennial forage breeding, in terms of genetic gain from selection, (ii) evaluate and predict the correlated response of desired traits in sward plots when selection is based on spaced-plant trials, under different genetic correlation and heritability scenarios, and (iii) describe and evaluate approaches to use modern BLUP-based selection methods for perennial forage breeding.

**MATERIALS AND METHODS**

Deterministic equations were derived for eight different selection methods applied to perennial forage species, aiming to estimate selection accuracy and selection gain. All methods were based on the use of half-sib families, based on three fundamentally different evaluation schemes: spaced plants, sward plots, or a combination of both spaced plants and sward plots (Fig. 1).

Five of these methods are commonly conducted on forage breeding research (Vogel and Pedersen, 1993; Casler and Brummer, 2008; Wilkins and Humphreys, 2003): individual selection (IND), among-half-sib-family selection (HS), within-family selection (WF), among-and-within-family selection (AWFS), and parental selection (PST). We have also considered the combined selection (CSBLUP) method, based on BLUP estimates of family performance when the experiments are balanced, a combination of HS and IND (HS–IND), and a combination of HS and combined selection methods (HS–CSBLUP). These three last methods are less commonly used in forage breeding and this is the first report of direct comparisons of these forage breeding strategies with some of the more traditional breeding methods.

**Details of Breeding Methods**

Consider the following generalized selection index as a method of expressing among-family and within-family phases of the selection process:

\[
I_g = \beta_1 \left( \bar{Y}_g - \bar{Y} \right) + \beta_2 \left( V_g - \bar{Y}_g \right),
\]
in the trait of interest (Casler, 2008). Family selection can also be conducted using remnant seeds, which is not a common practice for forage breeders due to the low inherent efficiency of selection. The principle of this method is identical to the HS method of Casler and Brummer (2008).

3) Within-family selection (WF):

Selection is based on choosing the best individuals of each family using the same intensity for each family. In this method, the effect of family is not considered but only the deviation of each individual in relation to family mean. Based on this aspect, this selection method should be applied in spaced-plant nurseries for traits that can be adequately evaluated under noncompetitive conditions.

4) Among and within family selection (AWFS), selection practiced in two stages: $b_1 = 1$ and $b_2 = 0$ in the first stage and $b_1 = 0$ and $b_2 = 1$ in the second stage. The best families are first selected in the sward-plot trial (Fig. 1) and, second, the individuals of each selected family are weighted by their own phenotypic value in the spaced-plant trial and the best individuals are selected only within the best families (Fig. 1). The fraction selected within family will be the same for each selected family. This method is equivalent to AWFS with recombination of selected plants within selected families described by Casler and Brummer (2008) and conceptually similar

Figure 1. Schematic flow diagram of eight half-sib family selection methods differing in selection unit and assuming 2 yr of data collection before selecting the best families and/or individuals for the target trait $y$. The selection method begins with the development of $N$ half-sib families, which are planted in field trials for phenotypic measurement of trait $y$. Individual selection (IND), within-family selection (WF), and combined-among-and-within families (combined selection [CSBLUP]) methods are based exclusively on spaced-plant trials, which are organized according to family structure. Among-half-sib-family selection (HS) and parental selection (PST) methods are based exclusively on sward-plot trials of half-sib families. Among-and-within-family selection (AWFS), the combination of HS with IND (HS–IND) selection, and the combination of HS with CSBLUP (HS–CSBLUP) all use both plot types as components of the selection system. In the latter three methods, sward plots are used to select among families and spaced plants are used to select within families.
to the between- and within-family selection method described by Vogel and Pedersen (1993).

5) Combined selection (CSBLUP): $\beta_1 = \{(1 - t)[1 + (n - 1) \rho_p]/[1 - t \rho_p(1 + (n - 1))])$ and $\beta_2 = 1$, in which $t$ is the phenotypic intraclass correlation coefficient, $t = \rho_{12}^2$, $\rho_p$ is equal to 0.25 for half-sibs (covariance of half-sibs equals 0.25 of the additive genetic variation), $h^2_i$ is the heritability of individual values, and $n$ is the number of individuals per family (Lush, 1947; Falconer and Mackay, 1996; Resende, 2002). Combined selection uses both the individual phenotypes and family information by using an optimal weighting scale for each source of information. This method differs from AWFS by selecting individuals with genetic merit that probably would not be selected by AWFS (for example, very good individuals belonging to average families).

6) A combination of methods HS and IND (HS–IND): The combination of methods of selection in forage breeding by recovering information of field trials is not a new idea. Vogel and Pedersen (1993) have suggested recurrent multistep family selection to select among and within half-sib families and to select a subset of superior genotypes from the parent polycross nursery using the means obtained from their replicated progeny. Methods HS–IND and HS–CSBLUP were proposed to explore all genetic information available when families are experimentally evaluated in sward plots and in spaced-plant plots (Fig. 1).

7) A combination of the methods HS and CSBLUP (HS–CSBLUP).

8) Parental selection (PST): $\beta_2 = 0$ and $\beta_1 = 2nh^2_i/n + (4-h^2_i)/h^2_i$, in which $(4-h^2_i)/h^2_i$ is constant for a given trait in a population. Parental selection requires that parental genotypes be saved during the progeny evaluation. Parents of the best families are polycrossed in isolation. This method is equivalent to the half-sib progeny test by Vogel and Pedersen (1993) and also was considered by these authors as one part of recurrent multistep family selection. This method permits the identification of elite genotypes, with good general combining ability, that can be used to produce synthetic cultivars or hybrids in the first cycle of selection, resulting in immediate selection gain. However, as stated by Vogel and Pedersen (1993), this method is usually stopped after a single cycle for the reason that repeating the process would involve the reevaluation of the same clones mated to a smaller number of parents. Population effective number is a serious concern of PST when breeding is continued over multiple cycles of selection.

### Deterministic Equations for Selection Gain

For IND the general equation for selection gain is given by $G_i = (k h \sigma_y)/T$ (Falconer and Mackay, 1996), in which $k$ is the intensity of selection, $h = \sigma_s/\sigma_y$, $\sigma_y$ is the phenotypic standard deviation, and $T$ is the unit of time required from planting the candidate individuals to selection and planting the progeny to begin the next selection cycle. So $T$ depends on plant age at the time of selection, time necessary to produce seeds, and the time necessary, after seed production, for new planting and completion of the cycle. Therefore, all efforts to minimize any components of $T$ will contribute to higher genetic gain.

The value of an individual under selection is equal to the sum of the average effects of the genes it carries, the summation being made over the pair of alleles at each locus and over all loci, generally termed the breeding value of the individual (Falconer and Mackay, 1996). Generally the predicted breeding value is not equal to the true breeding value of individuals. The correlation between predicted and true breeding values is generally less than 1.0 and is called accuracy (Van Vleck et al., 1987), symbolized here by $r_{a\hat{s}}$. Because accuracy is a statistic associated with the precision of selection in practice, this parameter is the most important in comparing selection methods. Expressing genetic gain in terms of accuracy is advantageous to compare selection methods because $\sigma_y^2$ is a property of the population and, consequently, is constant across selection methods. Higher accuracy values are indicative of better predicted breeding values (Falconer and Mackay, 1996) and more efficient selection method, provided $T$ and $k$ are kept constant (Resende, 2002).

Based on the above, a generalized equation for estimating genetic gain is $\hat{G}_i = (k h \sigma_y)/T$, in which $r_{a\hat{s}}$ is the accuracy of predicting breeding values according to the selection method to be used. The accuracy and genetic gain equations for the methods compared in this paper are shown on Table 1. Intensity of selection, $k$, was not considered constant across the methods we compared and details will be given later.

The accuracy of IND is $h$, the square root of heritability, because the selection criterion is the individual’s phenotypic value (Falconer and Mackay, 1996). The generalized estimator of accuracy was used for equations of other methods to be compared, according to Resende (2002) and Mrode and Thompson (2005): $r_{a\hat{s}} = [(\sum_{i=1}^{n} \beta_i a_{\hat{s}i})^{1/2} = (h \sigma_y + \beta_i^2 a_{\hat{s}i})^{1/2}$, in which $\beta_i$ is the weighting coefficient of the $i$th component of the index and $a_{\hat{s}i}$ is the additive correlation between the component $i$ of the index and the breeding value.

Selection reduces the genetic variance in the progeny, mostly by creating linkage disequilibrium (Bulmer 1971), even under the infinitesimal model. Estimated heritability tends to be lower than real heritability as a result of the Bulmer effect. Therefore, the phenotypic variance ($\sigma_y^2$) and additive variance ($\sigma_a^2$) of the original population are reduced by one factor $k^*$. Factor $k^*$ is dependent on the selection intensity and it is equal to $k^* = k/(k - d)$ (Falconer and Mackay, 1996), in which $k$ is the standardized selection differential ($k = 1.271$ for selection intensity $\alpha = 0.25$) and $d$ is the deviation of the truncation point relative to overall mean.

Therefore, the correcte variances are

$$0.25\sigma^2_{a^*} = \left[1 - \left(\frac{n(0.25)h^2_i k^*}{[1 + (n - 1)0.25h^2_i]}\right)\right] \left[0.25h^2_i \sigma_y^2\right].$$
### Table 1. Accuracy and genetic gain equations for eight methods of forage breeding based on phenotypic data.

<table>
<thead>
<tr>
<th>Method</th>
<th>Accuracy</th>
<th>Selection gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Individual selection (IND)</td>
<td>( r_{\text{IND}} = \left( \frac{h_s^2}{s} \right)^{1/2} )</td>
<td>( \hat{G}_s = \left( k h_s^2 \sigma_s / T \right) )</td>
</tr>
<tr>
<td>2. Among-half-sib-family selection (HS)</td>
<td>( r_{\text{aw}} = \left{ \frac{n (0.25)^2 h_s^2 / \left[ 1 + (n-1) 0.25 h_s^2 \right]}{0.25 h_s^2 + (1-0.25) h_s^2} \right}^{1/2} )</td>
<td>( \hat{G}<em>s = \left( k r</em>{\text{aw}} \sigma_s / T \right) )</td>
</tr>
<tr>
<td>3. Within-family selection (WF)</td>
<td>( r_{\text{aw}} = \left{ \frac{[(n-1) / n] \left[ 1 - 0.25 s \right] / \left[ 0.25 h_s^2 + (1-0.25) h_s^2 \right]}{0.25 h_s^2 + (1-0.25) h_s^2} \right}^{1/2} )</td>
<td>( \hat{G}<em>s = \left( k r</em>{\text{aw}} \sigma_s / T \right) )</td>
</tr>
<tr>
<td>4. Among-and-within-family selection (AWFS)</td>
<td>( r_{\text{aw}} = \left( \left{ \frac{k r_{\text{aw}} \sigma_s / T \right} \right) )</td>
<td>( \hat{G}<em>{aw} = \left( k r</em>{\text{aw}} \sigma_s / T \right) )</td>
</tr>
<tr>
<td>5. Combined selection (CSBLUP)</td>
<td>( r_{\text{aw}} = \left{ \frac{n (0.25)^2 h_s^2 / \left[ 1 + (n-1) 0.25 h_s^2 \right]}{0.25 h_s^2 + (1-0.25) h_s^2} \right}^{1/2} )</td>
<td>( \hat{G}<em>{aw} = \left( k r</em>{\text{aw}} \sigma_s / T \right) )</td>
</tr>
<tr>
<td>6. Among-half-sib-family selection (HS) plus individual selection (IND) on the mixture of families in the recombination experiment (HS–IND)</td>
<td>( r_{\text{aw}} + r_{\text{las}} )</td>
<td>( \hat{G}<em>{aw} = \hat{G}</em>{aw} + \hat{G}_{las} )</td>
</tr>
<tr>
<td>7. Among-half-sib-family selection (HS) in sward plots evaluation plus combined selection (CSBLUP) in the recombination field (HS–CSBLUP)</td>
<td>( r_{\text{aw}} + r_{\text{las}} )</td>
<td>( \hat{G}<em>{aw} = \hat{G}</em>{aw} + \hat{G}_{las} )</td>
</tr>
<tr>
<td>8. Parental selection (PST)</td>
<td>( r_{\text{aparental}} = \left{ \frac{n / \left[ n + (4 - h_s^2) / h_s^2 \right]}{0.25 h_s^2 + (1-0.25) h_s^2} \right}^{1/2} )</td>
<td>( \hat{G}<em>{aparental} = \left( 2 k r</em>{\text{aparental}} \sigma_s / T \right) )</td>
</tr>
</tbody>
</table>

### Experimental Conditions and Parameter Values

Several situations relevant to breeding forage and biomass crops were considered in this research. The selection criterion can be any agronomically important quantitatively inherited trait related to forage or biomass production. All models were based on selection for one trait and one cycle of selection.

1. Based on field experiments commonly conducted for obtaining phenotypic data on traits, we considered two different kinds of half-sib family evaluation, with selection in both sexes, perennial parents still alive and with remnant seeds, according to Casler (2010). First, each family was simulated as being established in sward plots, at a density of 400 plants m\(^{-2}\), planted in 0.9 by 1.4 m plots with five drilled rows spaced 15 cm apart. Second, each family was simulated as being established in a spaced-plant trial, using plots consisting of 10 plants spaced 0.3 by 0.9 m apart, allowing collection of phenotypic information on individual plants.
2. The total number of families evaluated in spaced-plant trials was 150, with 40 individuals per family, resulting in a total sample of 6000 individuals (\( N = 6000 \)). The experimental design was a randomized complete block with four replicates.
3. Common estimates of heritability in different forage species vary from very low to very high (\( h_s^2 = 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, \) and 0.7). Heritability estimates for model species, as switchgrass (\( \text{Panicum virgatum} \), fall within this range of values (Bhandari et al., 2010, 2011; Rose et al., 2008). For AWFS the same magnitude of

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\( h_s^2 = \) heritability of individual
\( \sigma_s^2 = \) corrected among-half-sib-family variance
\( \sigma_y^2 = \) corrected additive variance
\( n = \) number of individuals
\( h_s^2 = \) heritability of half-sib family
\( \sigma_s^2 = \) among-half-sib-family additive variance
\( \sigma_y = \) sib-family additive variance

\[ s_{aw} = \left\{ \frac{n (0.25)^2 h_s^2 / \left[ 1 + (n-1) 0.25 h_s^2 \right]}{0.25 h_s^2 + (1-0.25) h_s^2} \right\}^{1/2} \]

\[ s_{aw} = \left\{ \frac{[(n-1) / n] \left[ 1 - 0.25 s \right] / \left[ 0.25 h_s^2 + (1-0.25) h_s^2 \right]}{0.25 h_s^2 + (1-0.25) h_s^2} \right\}^{1/2} \]
Correlated Response

Because intergenotypic competition may impact trait expression and resultant breeding values, measurements of biomass yield made on spaced plants vs. sward plots should be considered as potentially different traits as suggested by Casler and Brummer (2008). This approach requires a correlated response approach to estimate selection gain for all the different methods studied, except HS. Correlated responses were estimated according Falconer and Mackay (1996) and Resende (2002) and given by

$$CR_x = kh_x \sigma_x, \quad CR_y = yh_y \sigma_y,$$

in which $h_x$ is the corrected within-family $k$ value and was equal to 1.692 in our study, $y$ is the proportion selected, $\sigma_x$ is the square root of heritability of character $x$ (secondary character, the selection criterion for spaced plants), $\sigma_y$ is the genetic correlation between the characters $x$ and $y$, and $\sigma_y$ is the genetic standard deviation of character $y$ (desired character, the evaluation criterion measured on sward plots). Accuracy for correlated responses is given by $h_x$ and the result of its application was used to substitute for $k_y$ in each equation in Table 1.

Based on efficiency of correlated response in relation to direct selection it is important to remember that even when the genetic correlation between $x$ and $y$ is equal to unit (1), it is still necessary that $h_x > h_y$ in order for indirect selection to be superior to direct selection. As this is not often the case in forage breeding practice (Riday and Brummer, 2004; Annicchiarico, 2006; Casler, 2008), the estimates of correlated responses will allow us to verify if there are differences between the various selection methods that might aid in dealing with these indirect selection issues. For the purpose of comparison among methods, the merit of each method was estimated as a relative efficiency in relation to selection gain based on the IND with accuracy $h_x$.

Correlated responses for trait $y$ were estimated for different combinations of narrow-sense heritability, estimated in the spaced-plant trial in which $h_x^2$ corresponds to various fractions (0.33, 0.5, 0.67, and 1.0) of the $h_y^2$ values of narrow-sense heritability estimated in the sward-plot trial ($h_x^2 = 0.2, 0.4, 0.6, 0.8$) and different genetic correlations between $x$ and $y$ ($r_{xy} = 0.3, 0.5, 0.7$ and 1). Biomass yield of switchgrass is a common example of an economic trait that is frequently the subject of selection on either spaced plants or sward plots, between which the genetic correlation may vary across populations and environmental conditions (Missaoui et al., 2005; Rose et al., 2008).

While genotype × environment (G×E) interaction is not in the scope of this paper, it can have significant impact on selection gains. Because the vast majority of forage breeding occurs at a single location and there are literally hundreds of examples in which breeding at a single location results in gains that are realized in multiple environments (e.g., Casler and Brummer, 2008; Rose et al., 2008; Wilkins and Humphreys, 2003), we chose to ignore G×E in our modeling exercise. A G×E component of a breeding program could be introduced into our models, but we would likely assume the G×E effects to be similar across breeding methods. The exceptions would be the breeding methods based on replicated plots, in which replicates or blocks could be distributed across multiple locations as a normal part of the selection procedure (e.g., Casler, 2010).

RESULTS

From the expressions for selection gain (Table 1), it is possible to estimate genetic gains by giving values to the parameters. Selection schemes can then be compared numerically for their relative efficiencies. Relative efficiency of the eight methods was expressed in relation to IND as a function of trait heritability (Fig. 2). The WF method was always lower in predicted gain in relation to IND, irrespective of the heritability value. The HS method was advantageous in relation to IND only when heritability was very low (0.01). The WF and HS methods are usually applied in evaluations based in spaced-plant trials and sward-plot trials, respectively, and were the most inefficient methods in terms of selection gain. The CSBLUP method was superior to IND when heritability was between 0.01 and 0.3, which is a common range of heritability for individual-plant evaluations of many quantitative traits. Accuracy of CSBLUP depends on family-mean and individual heritabilities, both weighted by the same intensity of selection $k_i$. Thus, as the heritability increases, the weight given for the component $\beta_1$ of the generalized selection index is reduced (the individual information turns out to be more important) and the relative response of CSBLUP is reduced in relation to IND.

There is a clear advantage in terms of selection gain when both plot designs are available (spaced plant and sward plots) within a selection cycle or generation since it enables the use of more than one method of selection simultaneously, increasing the gain and the accuracy selection. Parental selection and HS–CSBLUP are superior to the method HS–IND when heritability is low. As the heritability increases, a higher proportional gain is obtained when
the selection method HS–IND is practiced simultaneously in spaced plantings and sward plots. Because the relative differences between methods are proportionally reduced for higher trait heritability the choice of breeding method, especially the decision to use spaced plantings, sward plots, or both, is more critical for low-heritability traits.

Relative efficiency of correlated responses for each selection method is presented in Fig. 3. The IND and WF methods showed 50 to 60% loss of efficiency when the genetic correlation between trait $y$ in sward plots and trait $x$ in spaced planting is low ($r_{xyc} = 0.3$). That magnitude of loss in estimated gain was not dependent on the magnitude of heritability. Both CSBLUP and HS–CSBLUP methods were consistently higher in average correlated response, demonstrating reduced variability in response compared to all other methods, including the PST method. This advantage was generally greatest when trait heritability was low to moderate.

Combined selection in spaced plantings and sward plots (HS–CSBLUP) was the best method when heritability for the trait $y$ was low (0.2), even when the heritability for trait $x$ was only 0.33 of the heritability for trait $y$ (Fig. 4). Efficiency of HS–CSBLUP was consistently 40 to 90% higher than for IND method when heritability was low. The advantage of HS–CSBLUP over IND averaged about 18 to 46% for moderate heritability of trait $y$ (Fig. 4). However, for high heritability of trait $y$, efficiency of HS–CSBLUP was <100 relative to IND regardless of the magnitude of the genetic correlation (Fig. 4). Methods AWFS and HS–IND were the best methods for high genetic correlations and high heritability of trait $y$.

**DISCUSSION**

Forage breeding can be extraordinarily complex when compared with breeding annual crops, mostly because of the number of species, perenniality, mode of reproduction (Wilkins and Humphreys, 2003; Sokolović et al., 2011), and mating system (Santos-Garcia et al., 2011). Perenniality directly impacts the choice of breeding methods, creating more similarity to animal and forestry breeding methods than for annual crops. Perenniality also increases the average time required to complete a cycle or generation of selection, decreasing the number of recombination events that can be accomplished within a given length of time (Wilkins and Humphreys, 2003). Allogamy is the predominant mating system in forage grasses and legumes (Brummer, 1999; Riday and Brummer, 2002; Maureira et al., 2004; Abberton and Marshall, 2005; Yang et al., 2008; Walter et al., 2012).

Selection methods in forage breeding are largely based on open pollination, resulting in the development of half-sib progeny for evaluation (Posselt, 2010; Walter et al., 2012). In some cases evaluations are based on full-sibs originated from controlled pollination (Bolaños-Aguilar et al., 2001; Riday and Brummer, 2002) generally with a reduced number of parental genotypes. The choice of breeding method is generally based on reproductive biology of the species (allogamy, autogamy, or mixed system) and type of cultivar to be developed (synthetic, hybrid, apomictic genotype, or vegetatively propagated genotype), characteristics that are often directly related to each other (Resende, 2002; Gallais, 2003). Controlled crosses in forages are promoted mostly to amplify variability and trait combinations within species (Riday and Brummer, 2002; Resende et al., 2004; Martinez-Reyna and Vogel, 2008; Vogel and Mitchell, 2008) or between species (Miles, 2007) but also to study genetic phenomena such as heterosis, heritability, and trait dissection (Bolaños-Aguilar et al., 2001; Vogel and Mitchell, 2008; Anhalt et al., 2009; Tomaszewski et al., 2012). Use of hybrid cultivars in forages is generally limited to species for which individual genotypes can be propagated by apomictic seed or vegetative propagules (Miles, 2007; Hanna and Anderson, 2008). Controlled pollination between parental genotypes on a field scale requires strong self-incompatibility mechanisms and efficient methods to propagate parental genotypes (Vogel and Mitchell, 2008; Yang et al., 2008; Posselt, 2010).

Based in these aspects, we focused on half-sib progeny methods of selection in this paper. Although Fig. 1 shows the eight selection methods grouped according to the method of field evaluation, the results of this study suggest a slightly different grouping, based on expected gains, as follows: (i) methods based on one form of evaluation, either spaced plants or sward plots, IND, HS, WF, and CSBLUP, with intercrossing of selected progeny, (ii) methods based on a combination of spaced plants and sward plots, AWFS, HS–IND, and HS–CSBLUP, with intercrossing of selected progeny, and (iii) one method PST, based on saving parent plants from the previous generation for recombination.
The results in Fig. 1 clearly show the very different genetic potential of these three groups of methods, which provide increasing and higher genetic gains from group (i) to group (iii). The PST system selects between progeny-tested parents so its reliability (squared accuracy) doubles that for half-sib family selection. Methods making use of both forms of field evaluation also performed well because they use more information than is available for a single form of field evaluation.

Parental selection was usually the best method in terms of selection gain. Because of this advantage, the PST method often appears to be the ideal method of selection (Falconer and Mackay, 1996). However, PST is not the most parsimonious method, considering the effective population size required to ensure long-term selection gains. As mentioned by Casler (2008), selection intensities are often low for family-based selection methods, due to the limited number of families that can be effectively evaluated in selection programs. Use of the PST method results in a bottleneck for each selection cycle, caused by the need to polycross a small number of selected parents, increasing the rate of inbreeding for a long-term selection program (Han and Casler, 1999). This requirement creates the need for a second recombination event within each cycle of selection, lengthening the cycle time \( T \) to create a sufficient number of families to conduct the next cycle of selection. In addition, the increased rate of inbreeding, caused by reduced average effective population size (Han and Casler, 1999), creates limits to selection that may not be present in breeding methods based on recombination of progeny plants (Posselt, 2010). The PST method can be characterized as “backward” selection because intercrossing is accomplished from parental plants that represent the previous generation.

Unless the selected parents have high breeding value, for example, some parents are selected to overlap with the next generation, a common practice in breeding apomictic species, PST should not be used as a platform for long-term recurrent selection in forage breeding programs. In breeding apomictic forage species, recurrent selection for specific combination ability (Miles, 2007) is practiced by crossing male apomictic individuals with female sexual individuals to generate progeny populations that segregate for sexual- and apomixis and exhibit heterosis. Because apomictic parents cannot be pair-crossed, the sexual population serves as the source of females to be improved by recurrent selection (Resende et al., 2004) while superior apomictic males will be frequently used across several generations.

Concerning group (i), CSBLUP is the best choice in terms of genetic gain. This method uses two genetic
components from relatives (family and within family) increasing the accuracy of prediction of the breeding values from phenotypic values (Resende, 2002; Gallais, 2003) and the genetic gain, which is not the case for the other three methods in this class. Interestingly, by using the same field trials and the same data, selection based on CSBLUP can result in 1% to 70% higher gain for low heritability traits ($h^2_x \leq 0.3$) in relation the IND and WF methods. In terms of genetic gain, HS should never be performed as the only method in forage breeding.

In group (ii), HS–IND and HS–CSBLUP are better because the selection intensity for IND is higher than WF intensity ($k_i > k_w$). Furthermore, HS–CSBLUP is better than HS–IND because HS–CSBLUP uses three genetic components (among half-sib family plus [among plus within family]) while HS–IND uses two genetic components (among family plus within family). Interestingly, both methods can be applied in the same field nurseries and plots, but HS–CSBLUP results in 18% more selection gain than HS–IND when heritability is low (0.1).

Besides mode of reproduction and mating system, heritability of the trait under selection should be considered in choosing among selection methods. Efficiency and accuracy of the selection methods depend on the magnitude of heritability and the adequate use of all genetic information available in experiments.

The spectrum of heritability can be divided in two ranges for practical purposes: (i) low (0.01 to 0.30) or (ii) moderate (0.30 to 0.60). For low heritability, conclusions are largely as stated above, reinforcing the idea that CSBLUP should be used when selection is based on spaced plants alone. Among-half-sib-family selection and combined-selection (HS–CSBLUP) method should be used when selection can be based on both spaced plants and sward plots and heritability is low. The methods within groups (i) and (ii) are similar to each other and to PST for moderate heritability, creating more flexibility within the breeding program and lessening the potential impact of choosing a less efficient breeding method for reasons other than accuracy or efficiency.

![Figure 4. Relative response of various selection methods calculated in proportion to the individual selection (IND) method (direct selection on trait $y$) as a function of genetic correlation among traits ($r_{x,y}$) when individual narrow-sense heritability of trait $x$ ($h^2_x$) is a proportion of narrow-sense heritability of the trait $y$ ($h^2_y$). AWFS, among-and-within-family selection; CSBLUP, combined selection; WF, within-family selection.](image-url)
SUMMARY AND CONCLUSIONS

In summary, selection methods differ most when heritability is lower than 0.30, which coincides with the majority of the situations met by forage breeders. Narrow-sense heritability for economic traits frequently falls within this range, for example, 0.13 to 0.24 for biomass yield of switchgrass (Rose et al., 2008; Bhandari et al., 2010, 2011), 0.2 to 0.4 for biomass quality traits of switchgrass (Hopkins et al., 1993), and 0.2 to 0.4 for forage quality traits of smooth brome grass (Carpenter and Casler, 1990; Culvenor and Casler, 1999). Our results indicate that BLUP-based methods should receive more serious consideration by forage breeders, especially now that computing methods are more readily available. Besides the property of genetic superiority, demonstrated here and in studies of other species, the BLUP approach has several interesting practical and estimation issues (Lynch and Walsh, 1998; Bernardo, 2010). Methods based on BLUP analysis deal well with missing data and unbalanced designs as well as enabling the efficient combination of a large amount of unbalanced information spanning generations, sites, years, and harvests; BLUP-based methods have been successfully used in animal and forestry breeding (Wei and Borralho, 2000; Piepho et al., 2008; Heffner et al., 2009; Bernardo, 2010).

Phenotypic selection based on spaced-plant evaluations is used in forage breeding to access the within-family genetic variability and it increases the accuracy and genetic gain as shown here. However, selection experiments in perennial grasses and legumes have shown that genetic correlations between some traits, for example, biomass yield, evaluated in spaced plantings and sward plots ranges from negative to positive values, depending on species and target trait (Casler and Tageldin, 1996; Annicchiarico, 2006; Casler, 2008; Casler and Brummer, 2008; Wilkins and Humphreys, 2003). The optimal choice of selection methods was independent of whether selection was based directly on the target trait y or indirectly on trait x, relying on correlated responses to achieve gains in trait y.

The best methods in terms of relative efficiency are those based on a combination of spaced-plant and sward-plot evaluations. Within this group, HS–CSBLUP was superior for traits with low heritability and low genetic correlation between spaced plants and swards. Certainly, the cost may be high when family evaluation is based on both spaced-plant and sward-plot trials (Conaghan and Casler, 2011). With advances in genotyping technologies and associated reductions in the cost of genotyping, the use of genomic breeding values based on markers will become available as indirect selection criteria in economically important crop forages. As genomic selection is based on individuals more than families, phenotyping will serve primarily to train prediction models (Jannink et al., 2010) and accuracy of phenotypic assessments will be critical for realizing long-term selection gains in a breeding population. Genomic selection methods are ideally suited to breeding methods such as HS–CSBLUP because spaced-plant nurseries could be replaced by glasshouse-grown seedlings undergoing DNA marker evaluations, minimizing negative impacts on cycle time (T).

While we have ignored G×E interactions in the formal aspects of our modeling exercise, each of the breeding methods we have described is amenable to modification that would take G×E interactions into account during the breeding phase. For example, we describe both spaced-plant nurseries and sward plots of half-sib families that are structured in randomized block designs with replication of families. It would be a simple, if expensive, matter to replicate those designs across locations in addition to replication within locations, accounting for G×E interaction and improving estimates of family-mean performance (Casler, 2013). If it can be accomplished, logistically and financially, evaluation and selection at multiple locations will increase the probability of developing broadly adapted cultivars compared to selection at a single location (Barker et al., 1997).

References

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