The Journal of the Iowa Academy of Science: JIAS

Volume 114 | Number 1-4 Article 4

2007

Narrow Sense Heritability and Additive Genetic Correlations in Alfalfa subsp. falcata

Heathcliffe Riday
U.S. Dairy Forage Research Center

E. Charles Brummer
University of Georgia

Jour. Iowa Acad. Sci. 114(1,4):28–34, 2007

Narrow Sense Heritability and Additive Genetic Correlations in Alfalfa subsp. *falcata*

HEATHCLIFFE RIDAY[1,3] and E. CHARLES BRUMMER[2,4]

[1]U.S. Dairy Forage Research Center, USDA-ARS, Madison, Wisconsin 53706
[2]Department of Crop and Soil Science, University of Georgia, Athens, Georgia 30602

The complex genetics of autotetraploid alfalfa (*Medicago sativa* L.) make additive genetic variance component estimation difficult. Halfsib family variances often are used to estimate additive genetic variances and, by extension, narrow sense heritabilities and additive genetic correlations. These estimates contain a portion of the dominance variance. Using such calculations, in conjunction with parent-offspring covariance estimates, the dominance component can be separated from the additive genetic component. This is rarely done. This study reports average estimates across 30 populations, of both additive and dominance variance component estimates based on between halfsib family variance and parent-offspring covariance for biomass yield, plant height, regrowth, plant width, plant growth angle, vegetative density, and maturity during each of three harvests. We consistently found negative dominance variance estimates. Based on previous theory, this suggests epistatic interactions are a noticeable component of most traits measured. Assuming no epistasis leads to inflated narrow sense heritability estimates when compared with estimates based on parent-offspring regression. Assuming no epistasis and no dominance variance, weighted averages of additive genetic variance between halfsib family and parent-offspring effects revealed plant width and vegetative density additively correlated with biomass yield. Peak photoperiod maturity had a nonsignificant negative additive correlation with biomass yield. Plant height had no additive correlation with biomass, in contrast to the strong phenotypic correlation observed. Additive genetic correlations for the same traits measured during different harvests in most instances were highly correlated. On average, third harvest heritabilities were greatest. Our results suggest selecting plants based on later season performance (August – October) is most effective for Iowa environments.

INDEX DESCRIPTORS: Alfalfa, *falcata*, herirability, genetic correlations.

Medicago sativa subsp. *falcata* forms a heterotic pattern for forage biomass yield with elite subsp. *sativa* (i.e., purple flowered alfalfa) breeding germplasm (Riday and Brummer 2002a, 2005). Falcata germplasm, however, is generally unimproved and unselected for Iowa and Midwestern North American environments. Undesirable falcata traits for an intensive agricultural system include slower regrowth, early onset of autumn dormancy, and decumbent growth habit (Lesins and Lesins 1979, Riday and Brummer 2002b, 2004). Commercial realization of sativa-falcata hybrids will require the development of faster regrowth, more erect plant habit, and less autumn dormant falcata germplasm.

Studies have been conducted examining genetic correlations between winter hardiness and autumn height (e.g., Brummer et al. 2000). The genetic correlations of agronomic traits, such as height and regrowth, with forage biomass yield are unknown. Because alfalfa is harvested multiple times per year, the correlations among traits may vary throughout the year. Narrow sense heritability estimates of biomass yield in a three harvest system revealed that the lowest heritabilities are observed during the first harvest with the highest during third harvest (Riday and Brummer 2005). If genetic correlations between harvests for the same trait are high, then determining the harvest with more favorable heritability would enable selection to be conducted when the greatest genetic gain could be achieved.

In diploids, the broad and narrow sense heritability estimates are based on total genetic variance and additive genetic variance, respectively (Hallauer and Miranda 1988). In autotetraploids, variances and covariances of common mating designs can be expressed as functions of genetic variance components (Levings and Dudley 1963) using quantitative genetic models presented by Kempthorne (1969). In autotetraploid alfalfa, (*Medicago sativa* L.) narrow sense heritabilities have been reported for many traits, but in the majority of cases, these were based on the variances among halfsib families, which includes a dominance component [$h^2 = (\sigma^2_A + {}^1/_9\sigma^2_D)/\sigma^2_P$]. Using parent-offspring covariances in conjunction with among halfsib family variances allows separation of the additive and dominance variance components. Few studies in autotetraploids have tried to separate the dominance component found in the "narrow sense" heritability estimate from the additive component. The dominance variance estimate for forage biomass yield in 'Cherokee' alfalfa was negative, suggesting that dominance variance was not important (Dudley et al. 1969). We recently reported similar findings for forage biomass yield (Riday and Brummer 2005). Of four biomass yield measures (total yearly yield and first, second, and third harvest yield), none had positive dominance variance. In contrast, in an experiment based on intercrosses and clones of seven genotypes from seven different populations, four of six alfalfa seed yield traits had positive dominance variance components (Bolaños-Aguilar et al. 2001). The dominance variance of the other two traits was zero, but it is unclear if the estimates were negative and

[3] Mention of trademarks, proprietary products, or vendors are included for the benefit of the reader and do not imply endorsement or preferential treatment by the USDA-ARS.

[4] This journal paper of the Iowa Agric. Home Econ. Exp. Stn., Ames, IA, Project No. 6631, was supported by Hatch Act and State of Iowa funds.

Table 1. Additive and dominance variance component estimates based on between halfsib family variances (σ^2_{HS}) and parent-offspring covariances (σ_{PO}) for each of three harvests for biomass yield, plant height, regrowth, plant width, growth angle, vegetative density, and maturity at harvest. Additive variance was calculated assuming the presence and absence of dominance variance, the latter estimate being a weighted average between σ^2_{HS} and σ_{PO}.

Trait		σ^2_A	σ^2_D	$\sigma^{2\,a}_A$
		Variance Estimate (SE of estimate)		
Biomass Yield				
Year Total (g plant^{-1})		846 (341)[b]	−1026 (2542)	643 (166)
	1st	116 (62)	−192 (558)	84 (31)
Harvest	2nd	85 (54)	−1 (377)	84 (26)
	3rd	99 (32)	−178 (229)	64 (16)
Plant Height (cm)				
	1st	7.4 (5.4)	25.1 (63.5)	10.9 (2.5)
Harvest	2nd	10.4 (4.3)	−5.9 (39.8)	9.4 (2.2)
	3rd	13.3 (5.2)	0.3 (40.7)	13.4 (2.6)
Autumn		33.2 (7.8)	−68.6 (23.3)	12.8 (2.1)
Regrowth (cm)				
Spring		0.026 (0.010)	−0.068 (0.037)	0.007 (0.003)
	1st	2.87 (0.83)	−6.21 (2.92)	1.11 (0.26)
Harvest	2nd	2.36 (0.67)	−4.93 (2.60)	1.01 (0.23)
	3rd	2.13 (0.62)	−3.38 (2.19)	1.17 (0.19)
Plant Width (cm)				
	1st	12.8 (5.3)	−5.9 (45.2)	11.8 (2.6)
Harvest	2nd	11.3 (4.3)	−10.4 (27.3)	9.1 (2.0)
	3rd	12.0 (3.7)	−18.3 (17.4)	7.4 (1.5)
Growth Angle (deg°)				
	1st	38.3 (12.0)	−69.1 (80.7)	24.1 (5.7)
Harvest	2nd	23.7 (7.3)	−41.6 (40.3)	14.2 (3.2)
	3rd	23.1 (7.5)	−23.7 (42.1)	17.7 (3.3)
Vegetative Density (mg cm^{-3})				
	1st	0.064 (0.026)	−0.090 (0.161)	0.044 (0.012)
Harvest	2nd	0.052 (0.028)	−0.020 (0.174)	0.047 (0.013)
	3rd	0.051 (0.021)	−0.071 (0.165)	0.038 (0.010)
Maturity at Harvest (Score)				
	1st	0.113 (0.040)	−0.060 (0.319)	0.102 (0.020)
Harvest	2nd	0.032 (0.024)	0.090 (0.216)	0.047 (0.012)
	3rd	0.094 (0.041)	0.050 (0.396)	0.102 (0.021)

[a]Weighted average of σ^2_A based on σ^2_{HS} and σ_{PO} assuming σ^2_D equals zero.
[b]Standard error of variance estimate in parentheses.

reported as zero, a standard statistical practice, or if the estimates indeed were zero.

In addition to heritabilities, plant breeders need to be aware of unfavorable genetic correlations between traits of interest to select appropriately. A genetic correlation (r_A) is defined as the correlation among breeding values, meaning a genetic correlation based on additive trait genetic variances (σ^2_A) and covariances between traits (Falconer and MacKay 1996). In the alfalfa literature, "genetic correlations" (r_G) are sometimes based on trait genetic variances (σ^2_G) and covariances between traits (Brummer et al. 2000), and additive genetic correlations are based on halfsib family variances and covariances, which again include a dominance variance component.

The first objective of this study was to compare narrow sense heritability estimates for important agronomic traits based on variances among halfsib families and covariances of parents and offspring. The second objective was to compare additive and phenotypic correlations between agronomic traits based on halfsib family variances and covariances and parent-offspring covariances.

MATERIALS AND METHODS

Plant Material

A total of 107 genotypes from 30 populations (2 to 5 genotypes per population) was included in this experiment (Table 1). Four of 30 populations consisted of elite sativa breeding germplasm from Pioneer Hi-bred International (Des Moines, Iowa), Forage Genetics (two populations) (West Salem, Wisconsin), and 'Innovator +Z'. The remaining 26 of 30 populations were wild and semi-improved falcata populations from the Midwest and across Eurasia. Multiple populations, despite low genotype numbers per population, enabled us to determine average heritabilities and genetic correlations across populations. This allowed a greater inference space for the estimates, which in the case of genetic correlations, is especially advantageous as genetic correlations have been shown to vary widely in different populations (Falconer and MacKay 1996).

The 107 genotypes collected across all 30 populations were testcrossed by hand to the four elite sativa populations (testers) in

Table 2. Narrow sense heritability estimates for three harvests based on between halfsib family variances (h^2_{HS}), parent-offspring covariances (h^2_{PO}), and the weighted average between both measures (h^2_X) for biomass yield, plant height, regrowth, plant width, growth angle, vegetative density, and maturity at harvest. For the same traits the ratio of weighted average additive to total genetic variance is included ($\frac{\sigma^2_A}{\sigma^2_G}$).

Trait		h^2_{HS}	h^2_{PO}	h^2_X	$\frac{\sigma^2_A}{\sigma^2_G}$
		Ratio			
Biomass Yield (g plant^{-1})					
Year Total		0.36	0.24	0.31	0.41
	1st	0.20	0.11	0.17	0.26
Harvest	2nd	0.29	0.29	0.29	0.46
	3rd	0.44	0.22	0.35	0.51
Plant Height (cm)					
	1st	0.48	0.24	0.38	0.44
Harvest	2nd	0.66	0.34	0.49	0.62
	3rd	0.70	0.52	0.61	0.70
Autumn		2.89	1.17	1.44	1.44
Regrowth (cm)					
Spring		0.78	0.12	0.29	0.29
	1st	1.33	0.49	0.68	0.68
Harvest	2nd	1.16	0.46	0.65	0.65
	3rd	1.68	0.96	1.12	1.12
Plant Width (cm)					
	1st	0.33	0.29	0.32	0.42
Harvest	2nd	0.50	0.39	0.45	0.69
	3rd	0.89	0.53	0.66	0.95
Growth Angle (deg°)					
	1st	0.19	0.29	0.20	0.24
Harvest	2nd	0.29	0.25	0.28	0.38
	3rd	0.42	0.42	0.42	0.51
Vegetative Density (mg cm^{-3})					
	1st	0.45	0.28	0.37	0.71
Harvest	2nd	0.37	0.34	0.36	1.14
	3rd	0.33	0.21	0.29	0.58
Maturity at Harvest (Score)					
	1st	0.42	0.37	0.41	0.55
Harvest	2nd	0.24	0.35	0.27	0.39
	3rd	0.31	0.34	0.31	0.40
Average		0.65	0.38	0.46	0.61
SE[a]		0.41	0.39	0.32	0.37
Harvest[b]	1st	0.48	0.30	0.36	0.47
	2nd	0.50	0.35	0.40	0.62
	3rd	0.68	0.46	0.54	0.68

[a]Average standard error of individual heritability estimates.
[b]Average heritabilities for all traits measured around specific harvests.

the greenhouse during the autumn/winter 1999 to 2000 for a total of 428 cross entries. Of these 428 entries, 64 were sativa by tester and 364 were falcata by tester. The 428 entries were a subset of a larger experiment reported previously (Riday and Brummer 2005). Florets were not emasculated. Because of self-incompatibility most seed produced was expected to be cross-pollinated (Viands et al. 1988). To reduce the risk of self-fertilization or of crossing among plants of the same population, individual plant-to-plant crosses were made between genotypes (Riday and Brummer 2005). Seed from the 428 entries and cuttings of the 107 parental genotypes were germinated or grown in the greenhouse in spring 2000.

Field Design

Seedlings and cuttings were hand transplanted at the Agronomy and Agricultural Engineering Research Farm west of Ames, Iowa in a Nicollet loam soil (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) on 1 Aug 2000 and at the Northeast Research Farm south of Nashua, Iowa in a Readlyn loam (fine-loamy, mixed, mesic Aquic Hapludolls) on 8 Aug 2000. At each location, the field experiment was arranged in an augmented plot design consisting of 20 incomplete blocks of 40 plots each, for a total of 800 plots (Riday and Brummer 2005). Each plot consisted of 16 plants that were planted in a two by eight plant grid, with plants separated 30 cm within a plot and plots separated 75 cm on all sides.

Trait Evaluation

Harvests for biomass yield were taken on 3 June 2001, 24 July 2001, 11 Sep 2001, 30 May 2002, 13 July 2002, and 30 Aug 2002 in Ames and on 10 June 2001, 18 July 2001, 30 Aug 2001, 13 June 2002, 18 July 2002, and 12 Sep 2002 in Nashua using a flail type harvester equipped with an electronic data collection system. Concurrent with biomass harvests (no more than three days before harvest), maturity, plant width, and plant height were measured on each plant. Maturity was visually scored on a 1 = early vegetative to 9 = ripe seed pod scale (Kalu and Fick 1981). Plant width was measured on each plant in a plot from the center of the crown to the furthest horizontal natural growth point and averaged for a single plot value. Similarly, plant height was the average natural height of all plants in the plot. In addition to measurements concurrent with harvest, plant height was also measured approximately on a weekly basis from plant emergence in the spring until the first damaging frost in the autumn. Further detail on the data collection is provided in Riday and Brummer (2004, 2005).

Vegetative density and growth angle were derived from measurements taken at the time of each harvest (Riday and Brummer 2004). Vegetative density was calculated as the dry matter weight of 16 plants (i.e., the full plot) divided by the volume of the vegetative matter in the plot. Vegetative matter volume was estimated based on a 3-dimensional space generated from plant height, width, and plot layout. Growth angle was calculated as the arctangent of the plant height divided by the plant width.

Regrowth was calculated by averaging daily height measurements for the first 20 days after first, second, and third harvests. Daily height measurements were calculated based on the plant height data taken throughout the growing season (Riday and Brummer 2004). Spring regrowth was calculated using the same method for regrowth, except that the average height was based on the first 20 days after plant emergence in the spring. Autumn asymptotic height was also calculated using the method for regrowth, except the average height was based on the 32nd to 52nd day after third harvest.

To keep computation at a manageable level, least squared means for each entry were calculated for each location-year combination, eliminating incomplete blocking effects, for bio-

mass yield, plant height, plant width, growth angle, vegetative density, and maturity at harvest. For the four regrowth measures and for autumn height, all calculations were done on an experiment wide basis.

Heritability Estimation

Among halfsib family variance ($\sigma^2_{HS} = 1/4\sigma^2_A + 1/36\sigma^2_D$), adjusting for fixed populations, was estimated based on Levings and Dudley (1963). To simplify computer calculations, location-year combinations were considered as environments. Testers (T) and environments (E) were considered fixed effects, and genotypes (σ^2_{HS}) and genotype interactions (σ^2_{HSxT}, σ^2_{HSxE}, and σ^2_{HSxTxE}) were random effects. All random effects were estimated directly using Proc Mixed (SAS, 2000). Variance among parental clones ($\sigma^2_C = \sigma^2_G$) was estimated by adjusting for fixed populations (Levings and Dudley 1963). Genotypes (i.e., σ^2_C) and genotype x environment interaction (σ^2_{CxE}) were considered random effects. Finally, parent-offspring covariances ($\sigma_{PO} = 1/2\sigma^2_A + 1/6\sigma^2_D$) were estimated based on testcross progeny means and genotypic clonal performance, adjusting for fixed populations (Levings and Dudley 1963). An analysis of covariance (Nguyen and Sleper 1983) was accomplished with environments as fixed effects and genotypes (σ_{PO}) and genotype x environment interactions (σ_{POxE}) as random effects using an adapted Proc Mixed program (Zamudio and Wolfinger 2002 especially appendix A, Holland 2005). The Asycov option in Proc Mixed was used to estimate variances around the estimates ofσ^2_{HS}, σ^2_G, and σ_{PO}. Based on σ^2_{HS} and σ_{PO}, the additive variance was estimated as ($\sigma^2_A = 6\sigma^2_{HS} - \sigma_{PO}$) and the dominance variance as ($\sigma^2_D = 9\sigma_{PO} - 18\sigma^2_{HS}$).

Three narrow sense heritability estimates were calculated and compared based on: (i) halfsib family variance estimates [$h^2_{HS} = (\sigma^2_A + 1/9\sigma^2_D)/\sigma^2_P$], (ii) parent-offspring covariance estimates [$h^2_{PO} = (\sigma^2_A + 1/3\sigma^2_D)/\sigma^2_P$], and (iii) the weighted average of halfsib family variance and parent-offspring covariance estimates $[h^2_{\bar{x}} = \left(\left(\frac{\sigma^2_{HS}}{4V(\sigma^2_{HS})} + \frac{\sigma_{PO}}{2V(\sigma_{PO})}\right) \Big/ \left(\frac{1}{16V(\sigma^2_{HS})} + \frac{1}{4V(\sigma_{PO})}\right)\right) \Big/ \sigma^2_P]$ ($V(\sigma^2_{HS})$ = variance of σ^2_{HS}; and $V(\sigma_{PO})$ = variance of σ_{PO}). The ratio of additive to total genetic variance was estimated as well, using the weighted average additive variance and the variance among clones. Standard errors around the heritability estimates were estimated according to Hallauer and Miranda (1988).

Genetic Correlations

Additive genetic correlations (r_A) (Falconer and MacKay 1996) were estimated based on additive variances and covariances estimates calculated from halfsib family variances, parent-offspring covariances, and their weighted average variances and covariances, adjusting for fixed populations. Simple genetic correlations (r_G) were estimated based on variances and covariances among clones, adjusted for fixed populations. A phenotypic correlation (r_P) was estimated in the same way as r_G, except that no adjustment for fixed populations was made. Pairwise trait covariances were estimated directly using a Proc Mixed program adapted from Holland (2005). The SAS code for the analysis of the covariance between two parent-offspring covariances for two different traits, adjusting for fixed populations, is presented in the Appendix.

The Asycov option in Proc Mixed was used to estimate variances around the variance and covariance estimates. Additive and dominance covariances were calculated in an analogous manner to their variances. Correlations were then estimated as the covariance between two traits divided by the square root of the variance of the two traits. Standard errors around the correlations were estimated with the delta method using IML code in SAS (Holland 2005).

RESULTS AND DISCUSSION

Inference Space

Estimates of quantitative genetic parameters are usually based on a single 'reference' population. It is important to note that in this study there are 30 reference populations and all estimates reported are the average values obtained by taking the mean of the 30 individual population estimates. And although each population was represented by only two to five plants, statistical power was gained by averaging across the 30 populations after accounting for specific population effects. Since the 30 reference populations represent a random sample of alfalfa subsp. *falcata* populations, the results of this study apply in a general manner to the *falcata* subspecies, although specific populations within *falcata* may vary from the general pattern.

Variance Component Estimates

We first estimated the additive and dominance variance components for traits based on halfsib family variances and parent-offspring covariances. For 20 of the 24 trait estimates, dominance variance estimates were negative (Table 1). This suggests that for most traits in autotetraploid alfalfa, dominance variance is negligible or that the estimate is biased. Negative dominance variance estimates were observed because $4\sigma^2_{HS} > 2\sigma_{PO}$. Dudley et al. (1969) made this same observation in trying to estimate biomass yield variance components, but Bolaños-Aguilar et al. (2001) did not observe this phenomenon in two thirds of seed development traits they measured. Given the large number of assumptions underlying autotetraploid variance component estimates, it is perhaps expected that the models produce unexpected results. Of the four dominance variance estimates that were positive in our experiment, two were for plant maturity at harvest, which was based primarily on floral development stage and is supported by Bolaños-Aguilar et al. (2001) who observed positive dominance in many seed development traits which is associated with flower development.

The dominance variance estimates had large standard errors (Table 1), but the consistently negative estimates suggest some type of bias. In the presence of epistasis, parent-offspring covariance gives a better estimate of additive genetic variance than does halfsib variance because the parent-offspring covariance is unaffected by linkage (Nguyen and Sleper 1983). Even with linkage disequilibrium, linkage ("position") effects due to loci being in close proximity will cause an upward bias in epistatic variance effects among halfsib families but not in parent-offspring covariances (Cockerham 1956). Additive × additive epistatic effects contained in the additive variance estimate under the assumption of no epistasis causes the bias. We therefore conclude that epistasis has a significant role for most quantitative traits.

Due to the epistatic bias, determining which mating design (halfsib family or parent-offspring) provides the better estimate of additive variance is not possible. One estimation method cannot be said to be more accurate than the other without assuming that either dominance or epistatic interactions are more important. Since $4\sigma^2_{HS}$ has less dominance variance than $2\sigma_{PO}$, we expect $4\sigma^2_{HS} < 2\sigma_{PO}$. Yet in the presence of linkage, theory predicts that epistatic variance will be inflated in σ^2_{HS} but not in σ_{PO}, leading to the expectation that $4\sigma^2_{HS} > 2\sigma_{PO}$. For practical purposes, we assumed no dominance and no epistasis to determine the weighted average of σ^2_A, based on σ^2_{HS} and σ_{PO} (Table 1).

Heritability estimates

A paired t-test revealed heritabilities based on between halfsib family variances (h^2_{HS}) were significantly greater on average than heritabilities generated from parent-offspring covariances (h^2_{PO}) ($p = 0.0036$; Table 2). In autotetraploids, a halfsib mating design will have higher heritability estimates compared to those based on parent-offspring regression design if epistasis is present. Heritabilities for traits measured during third harvest tended to be greater than heritabilities measured earlier in the growing season (Table 2). Biomass yield, growth angle, vegetative density, and maturity had heritabilities ≤ 0.40 during all harvests. Plant height, plant width, and regrowth had heritabilities in the 0.30 to 0.70 range (Table 2). For autumn height and regrowth after third harvest, the heritability exceeded one. These spurious results are likely due to autumn dormancy effects. In autumn, falcata genotypes had entered almost total dormancy and had virtually no autumn regrowth or growth (i.e., $\sigma^2_A > \sigma^2_G$). The intersubspecific testcross progeny showed greater variance than their parental clones, which were mostly falcata, and this resulted in the observed heritability estimates.

Genetic Correlations

After generating all pairwise genetic correlations based on halfsib families (r_{HS}), parent-offspring regression (r_{HS}), parental clones (r_G), and parental clones unadjusted for populations (r_P), the four sets of correlations were compared. More similar correlations were r_{HS} with r_{PO} ($r = 0.87$) and r_{PO} with r_G ($r = 0.86$). The other correlation measures were also correlated with each other ($r = 0.71$ to 0.77), with the correlation of r_{HS} with r_P being most dissimilar ($r = 0.71$). Based on these results we used the weighted average variances and covariances calculated from halfsib families and parent-offspring regression to determine the additive genetic correlation (r_A), which we compared with r_P.

Biomass yield variables had high additive genetic correlations with each other ($r_A > 0.90$) except for first and third harvest ($r_A = 0.76$) (Table 3). The high r_A values suggest that selection based on data recorded any time during the year should improve biomass yield throughout the year. The r_A for plant height among harvests were correlated with each other ($r_A = 0.61$ to 0.87) (Table 3). Autumn height, however, showed only minor correlations with other height measures. Spring regrowth was not correlated with regrowth measured after harvesting. Regrowth after harvests wete correlated with each other ($r_A = 0.69$ to 0.84). This suggests that early vigorous spring emergence is not indicative of regrowth ability after cuttings. Plant width, growth angle, and vegetative density between harvests had strong additive genetic correlations with one another (Table 3). Maturity at second harvest had strong correlations with first and third harvest maturity ($r_A = 0.93$ and 0.74), while first and third harvest maturity had a weaker correlation ($r_A = 0.59$) (Table 3). With the notable exceptions of autumn height and spring regrowth, most traits had high additive correlations across differing harvests, suggesting that selecting for these traits during one harvest will result in concurrent improved trait performance during other harvests.

No additive genetic correlations were observed between biomass yield and plant height, with the exception of third harvest yield being weakly correlated with second and third harvest plant height ($r_A = 0.43$ and 0.44, respectively). The smaller correlation was not expected because the phenotypic correlations between plant height (except autumn height) and yield during all harvests were moderately strong ($r_A = 0.60$ to 0.79) (Table 3). This suggests that selection for height alone would not lead to increased biomass. These measurements, however, were taken on semi-sward plots (2×8 plants, with 30 cm between plants). If a similar study was conducted in a sward, an additive genetic correlation between plant height and biomass yield may occur. Our planting is more reminiscent of a mixed species hay or pasture situation where we would expect our correlation matrix to be applicable. This experiment also was conducted under a three harvest per year management; more frequent cuttings may change the correlations of biomass yield with plant height and possibly regrowth.

Yield and plant width showed moderate additive genetic correlations for almost all combinations ($r_A = 0.45$ to 0.72) (Table 3). No additive genetic correlation was observed for any yield by plant growth angle combination, but moderate phenotypic correlations were observed for these traits ($r_P = 0.42$ to 0.61). Yield was correlated with vegetative density (both r_A and r_P). In previous studies increased crown size and greater number of crown buds per crown correlated with yield (Marquez-Ortiz et al. 1996, Kimbeng and Bingham 1998). Visual observation of plots, in our study, suggested that the greatest vegetative density was usually derived from plants with dense growth resulting from many stems on large and spreading crowns.

Third-growth period regrowth, measured after second harvest in mid July, had additive genetic correlations with yield measures ($r_A = 0.50$ to 0.59). Phenotypic correlations between total yearly and second harvest biomass yield and regrowth measures and third growth period regrowth and all yield measures were moderate ($r_P = 0.40$ to 0.50) (Table 3). Weak negative additive genetic correlations were observed between maturity at second harvest and yield ($r_A = -0.42$ to -0.51) (Table 3). A photoperiodic effect seems to be active about the time of maximum day length (June 21) because early maturity during second harvest is negatively associated with biomass accumulation. The rapid regrowth following the second harvest shows an additive genetic correlation with biomass yield, which also may be related to photoperiod.

The regrowth measures had varying additive genetic correlations with plant height (Table 3). A weak negative r_A was observed between spring regrowth and third harvest height ($r_A = -0.48$). Weak r_A were also measured between third regrowth period and second ($r_A = 0.43$) and third harvest heights ($r_A = 0.44$). Very strong additive genetic correlations were observed between autumn plant height and second through fourth regrowth periods ($r_A = 0.95$ to 0.97). Phenotypic correlations between regrowth measures were moderately strong and consistently positive between second through fourth regrowth periods and all height measurements ($r_P = 0.62$ to 0.86). Strong additive genetic and phenotypic correlations were observed between plant height and growth angle combinations, but not

Table 3. Additive (above diagonal) and phenotypic (below diagonal) correlations for each of three harvests for biomass yield, plant height, regrowth, plant width, growth angle, vegetative density, and maturity at harvest.

		Biomass Yield				Plant Height				Regrowth				Plant Width			Growth Angle			Vegetative Density			Maturity at Harvest		
Harvest		Tot.	1st	2nd	3rd	1st	2nd	3rd	Aut.	Spr.	2nd	3rd	4th	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Biomass Yield	Tot.		0.97	1.00	0.92	ns	ns	ns	ns	ns	ns	0.50	ns	0.50	0.59	0.71	ns	ns	ns	0.62	0.68	0.81	ns	-0.42	ns
	1st	[a]0.98		0.99	0.76	ns	ns	ns	ns	ns	ns	ns	ns	0.64	0.62	0.72	ns	ns	ns	0.52	0.72	0.81	ns	ns	ns
	2nd	0.96	0.92		0.91	ns	ns	ns	ns	ns	ns	0.50	ns	0.45	0.65	0.72	ns	ns	ns	0.77	0.76	0.83	-0.40	-0.51	ns
	3rd	0.95	0.92	0.86		ns	0.44	0.43	ns	ns	ns	0.59	ns	ns	0.46	0.61	ns	ns	ns	0.47	0.48	0.64	ns	-0.43	ns
Plant Height	1st	0.72	0.79	0.62	0.65		0.61	0.70	ns	ns	ns	ns	ns	ns	ns	ns	0.91	0.90	0.83	ns	ns	ns	ns	ns	ns
	2nd	0.72	0.75	0.65	0.67	0.97		0.87	0.44	ns	ns	0.44	ns	ns	ns	ns	0.92	0.95	0.92	ns	ns	ns	ns	ns	ns
	3rd	0.66	0.66	0.60	0.64	0.91	0.97		0.51	-0.48	ns	0.43	ns	ns	ns	ns	0.90	0.92	0.95	ns	ns	ns	ns	ns	ns
	Aut.	0.43	ns	0.46	ns	0.71	0.79	0.85		ns	0.95	0.95	0.97	ns	ns	ns	0.74	0.70	0.81	ns	ns	ns	ns	ns	ns
Regrowth	Spr.	ns	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	2nd	0.40	ns	0.44	ns	0.62	0.74	0.79	0.68	ns		0.84	0.69	ns	ns	ns	0.67	0.65	0.75	ns	ns	ns	ns	ns	0.40
	3rd	0.50	0.43	0.49	0.46	0.73	0.81	0.86	0.77	ns	0.93		0.77	ns	ns	ns	0.76	0.72	0.82	ns	ns	ns	ns	ns	ns
	4th	0.42	ns	0.45	ns	0.65	0.74	0.80	0.86	ns	0.94	0.95		ns	ns	ns	0.69	0.65	0.77	ns	ns	ns	ns	ns	ns
Plant Width	1st	0.42	0.41	0.45	ns	ns	ns	ns	ns	ns	ns	ns	ns		0.91	0.84	-0.82	-0.75	-0.62	ns	ns	0.41	ns	ns	ns
	2nd	ns	ns	0.46	ns	ns	ns	ns	ns	ns	ns	ns	ns	1.11		1.00	-0.67	-0.73	-0.61	ns	ns	0.44	ns	ns	ns
	3rd	0.58	0.56	0.54	0.59	ns	ns	ns	ns	ns	ns	ns	ns	0.88	0.93		-0.68	-0.76	-0.65	ns	ns	0.50	ns	ns	ns
Grwth. Angle	1st	0.51	0.58	0.42	0.47	0.62	0.40	0.57	0.62	ns	0.55	0.47	ns	-0.43	-0.54	ns		0.77	0.83	ns	ns	-0.41	ns	ns	ns
	2nd	0.55	0.61	0.44	0.53	0.49	0.64	0.67	0.47	ns	0.53	ns	ns	-0.46	-0.52	ns	0.98		0.90	-0.47	ns	-0.46	ns	ns	ns
	3rd	0.50	0.52	0.44	0.49	0.48	0.62	0.79	0.73	ns	0.60	0.49	0.45	-0.48	-0.52	ns	0.94	0.96		ns	ns	-0.43	ns	ns	0.41
Veg. Dnsty.	1st	0.65	0.63	0.64	0.59	-0.61	-0.47	-0.49	ns	ns	ns	ns	0.50	ns	ns	0.41	ns	ns	ns		0.96	1.03	ns	ns	-0.42
	2nd	0.51	0.46	0.60	0.41	-0.43	-0.41	-0.41	ns	ns	ns	ns	ns	0.60	0.76	0.60	ns	-0.43	ns	0.87		0.87	ns	ns	ns
	3rd	ns	ns	0.41	0.43	-0.51	ns	ns	ns	ns	ns	ns	ns	0.72	0.62	0.81	-0.40	-0.41	-0.51	0.66	0.93		ns	ns	-0.40
Mat. at harvest	1st	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.50	ns	ns	ns	ns	ns	ns	ns		0.93	0.59
	2nd	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.42	ns	0.78		0.74
	3rd	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.69	

[a]SE for r_A = 0.18 and r_P = 0.11. Only correlations 0.40 or greater are reported.

between plant height and vegetative density, in which some phenotypic correlations were negative.

Regrowth for the second through fourth growth periods showed strong additive genetic and phenotypic correlations with growth angle, with r_A being the stronger of the two. Strong negative additive genetic correlations were observed between growth angle and plant width. Phenotypic second and third harvest vegetative density was correlated with plant width (r_P = 0.60 to 0.81). However, for additive genetic correlations only the third harvest vegetative density was weakly correlated with plant width. Third harvest vegetative density by growth angle combinations had weak negative correlations for both r_A and r_P (r_A = −0.41 to −0.46; r_P = −0.41 to −0.50). Plant growth angle is seemingly genetically associated with regrowth and dormancy, and more upright plants have better regrowth. The lack of correlation between plant height and regrowth indicates that plant size *per se* is not associated with faster regrowth; however, autumn height (i.e., dormancy) is strongly correlated with rate of regrowth.

Implications

The common assumption of no epistasis leads to inflated narrow-sense heritability estimates when using the among halfsib family variance as opposed to calculating the estimates based on parent-offspring regression. From a practical standpoint, we cannot recommend any easy solutions. A more complex mating design could be used, as discussed in Levings and Dudley (1963), but they emphasize the difficulties of conducting such an experiment. They suggest that parent-offspring regression would result in the best estimator of narrow sense heritability because the among halfsib family variance estimates would be 'under-estimated' (we observed the opposite). We agree with Levings and Dudley (1963) that parent-offspring regression would be a better estimator of narrow-sense heritability, except for the reason that it is more conservative.

Additive genetic correlations for the same traits measured during different harvests were significant in most cases. On average, third harvest heritabilities were greatest. These two results suggest selecting plants based on later season performance (August to October) is more effective for Iowa environments, at least for this falcata based germplasm. Biomass yield showed positive additive genetic correlations with plant width and vegetative density. This suggests, in conjunction with previous research, that visual selection based on crown size and stem crown density is more likely to lead to increased yield. Rapid maturity during peak photoperiod (late June) had a slight negative additive correlation with yield, but this may be related to the rather lax harvest management we imposed. Plant height showed no correlation with biomass yield.

ACKNOWLEDGMENTS

We thank the Raymond F. Baker Center for Plant Breeding for funding this research.

LITERATURE CITED

BOLAÑOS-AGUILAR, E. D., C. HUYGHE, D. DJUKIC, B. JULIER, and C. ECALLE. 2001. Genetic control of alfalfa seed yield and its components. Plant Breeding 120:67–72.

BRUMMER, E. C., M. M. SHAH, and D. LUTH. 2000. Re-examining the relationship between fall dormancy and winter hardiness in alfalfa. Crop Science 40:971–977.

COCKERHAM, C. C. 1956. Effects of linkage on the covariances between relatives. Genetics 41:138–141.

DUDLEY, J. W., T. H. BUSBICE, and C. S. LEVINGS, III. 1969. Estimates of genetic variance in 'Cherokee' alfalfa (*Medicago sativa*, L.). Crop Science 9:228–231.

FALCONER, D. S. and T. F. C. MACKAY. 1996. Introduction to quantitative genetics. Longman, Essex, England.

HALLAUER, A. R. and J. B. MIRANDA. 1988. Quantitative genetics in maize breeding. Iowa State University, Ames.

HOLLAND, J. B. 2005. Example SAS programs for estimating genotypic and phenotypic correlations and their standard errors. Retrieved 25 Mar 2005 from: http://www4.ncsu.edu/%7Ejholland/correlation/correlation.html

KALU, B. A. and G. W. FICK. 1981. Qualifying morphological development of alfalfa studies of herbage quality. Crop Science 21:267–271.

KEMPTHORNE, O. 1969. An introduction to genetic statistics. Iowa State University, Ames.

KIMBENG, C. A. and E. T. BINGHAM. 1998. Forage yield potential of alfalfa plant of varying crown size from old stands. Plant Breeding 117:251–254.

LESINS, K. and I. LESINS. 1979. Genus *Medicago* (Leguminasae): A taxogenetic study. Kluwer, Dordrecht, Netherlands.

LEVINGS, C. S. and J. W. DUDLEY. 1963. Evaluation of certain mating designs for estimation of genetic variance in autotetraploid alfalfa. Crop Science 3:532–535.

MARQUEZ-ORTIZ, J. J., L. D. JOHNSON, D. K. BARNES, and D. H. BASIGALUP. 1996. Crown morphology relationships among alfalfa plant introductions and cultivars. Crop Science 36:766–770.

NGUYEN, H. T. and D. A. SLEPER. 1983. Theory and application of half-sib matings in forage grass breeding. Theoretical Applied Genetics 64:187–196.

RIDAY, H. and E. C. BRUMMER. 2002a. Forage yield heterosis in alfalfa. Crop Science 42:716–723.

RIDAY, H. and E. C. BRUMMER. 2002b. Heterosis of Agronomic Traits in Alfalfa. Crop Science 42:1081–1087.

RIDAY, H. and E. C. BRUMMER. 2004. Morphological variation of *Medicago sativa* subsp. *falcata* genotypes and their hybrid progeny. Euphytica 138:107–112.

RIDAY, H. and E. C. BRUMMER. 2005. Heterosis in a broad range of alfalfa germplasm. Crop Science 45:8–17.

SAS INSTITUTE. 2000. SAS language and procedure: Usage. Version 8, 1st ed. SAS Inst., Cary, North Carolina.

VIANDS, D. R., P. SUN, and D. K. BARNES. 1988. Pollination control: Mechanical and sterility. Pages 931–960. *In* A. A. Hanson, D. K. Barnes, and R. R. Hill, Jr. (eds.). Alfalfa and alfalfa improvement. ASA-CSSA-SSSA, Madison, Wisconsin.

ZAMUDIO, F. and R. D. WOLFINGER. 2002. Growth increments and stability over time in fast-growing forest tree species. Canadian Journal of Forest Research 32:942–953.

APPENDIX

```
Proc Mixed Asycov;
Class Trait Pop Env PO Gen;
Model Y = Trait Pop(Trait) Env(Trait) Pop*Env(Trait)
          PO(Trait) Pop*PO(Trait) PO*Env(Trait)
          Pop*PO*Env(Trait);
Random Trait/subject = Gen(Pop) Type = UN;
Random Trait/subject = Env*Gen(Pop) Type = UN;
Random Trait/subject = PO*Gen(Pop) Type = UN;
Repeated Trait/subject = PO*Env*Gen(Pop) Type = UN;
Where,
Trait = trait 1 or 2;
Pop = population 1 to 30;
Env = environment 1 to 4;
PO = parent or offspring;
Gen = genotype 1 to 107.
```