Selection for Silage Quality in the Wisconsin Quality Synthetic and Related Maize Populations

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ABSTRACT

Maize (*Zea mays* L.) silage is a high-quality forage for ruminants, but there have been few significant breeding efforts specifically dedicated to improving maize forage yield or quality by breeders in the USA. The objective of this study was to evaluate the forage yield and quality of the Wisconsin Quality Synthetic (WQS) and related populations developed by the University of Wisconsin Maize Breeding Program for agronomic and nutritional attributes. Three cycles of divergent S_1 recurrent selection have been completed for stover fiber, silica, and lignin concentration for populations WFISIHI (Wisconsin-fiber-silica-high) and WFISILO (Wisconsin-fiber-silica-low). The third cycle (C3) of WFISILO was crossed to two high-quality inbred lines, Mo17 and H99, to create WQS C0, which then underwent two cycles of S_2–topcross selection for improved forage yield and quality. All cycles of selection for WFISIHI, WFISILO, and WQS were evaluated for forage yield and quality at two field locations in Wisconsin in 2000 and 2001. Results for WFISIHI and WFISILO demonstrated that S_1 selection for stover composition altered both stover and whole-plant composition in the anticipated directions. Selection for whole-plant yield and composition of S_2–topcrosses was also effective for WQS, especially when using selection indices incorporating whole-plant yield, neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), crude protein (CP), and starch. WQS C2 whole-plant and stover in vitro true digestibility (IVTD), whole-plant and stover acid detergent lignin, and milk yield per megagram dry matter (DM) were similar to the brown-midrib check hybrid, F657. WQS C2 whole-plant and stover NDFD were lower than F657 but higher than other commercial check hybrids. In addition, WQS population testcrosses improved over cycles of selection for whole-plant yield and most quality attributes. Our results indicate that it is feasible to develop silage maize germplasm with both high whole-plant yield and excellent nutritional quality.

THERE HAS BEEN little breeding effort to improve maize forage yield or quality in the USA (Lauer et al., 2001). The existence of genetic variation for forage nutritive quality in maize has been shown previously (Barrière and Argillier, 1998; Argillier et al., 1995, 2000). Barrière and Argillier (1998) stated that the specific breeding of maize for forage quality started 15 to 20 yr ago, albeit with germplasm previously selected for grain yield. Barrière and Argillier (1998) further suggested that to produce a silage maize that possesses high yield, high quality, and high lodging resistance, the breeders must utilize digestibility analyses in their breeding program. Coors and Lauer (2001) suggested there are two approaches to increasing total energy content through (i) increasing the contribution of grain and (ii) increasing the digestibility of the stover. Much of the variation in forage digestibility can be accounted for by differences in rates of fiber digestion and passage through the gut (Waldo et al., 1972). Ruminal function and animal health are optimal in forage-based diets (Jung and Allen, 1995), and performance depends on intake of digestible and metabolizable nutrients (Mertens, 1994). The importance of digestibility of the fiber was apparent in a study conducted by Muller et al. (1972) who fed lambs both brown-midrib and normal maize forages that were ensiled without the ears. The NDF concentrations of the hybrids were all equal, but NDFD was 20% greater in the brown-midrib forage. This resulted in 29% more DM intake by lambs fed the brown-midrib hybrid compared with the normal hybrid. Differences in the brown-midrib hybrid not only affected digestibility but also intake resulting in a substantially greater intake of digestible energy. In more recent studies, Oba and Allen (1999, 2000a, 2000b) found that brown-midrib maize silage that includes the *brown-midrib3* (*bm3*) gene had high dry matter intake and milk yield. The effects of the *bm3* gene are most evident on productivity in cows fed high NDF diets in conjunction with the *bm3* maize silage (Oba and Allen, 2000a). Further, Oba and Allen (1999, 2000b) stated that, although enhanced in vitro NDFD was associated with increased milk production, NDFD appeared to more directly affect rate of passage and dry matter intake rather than overall energy concentration. Tyrrell and Moe (1975) stated that as intake of less digestible forage increases, the efficiency of digestion may decrease. Because of the increased effort to improve the genetic potential for growth and lactation of ruminants, there is a need for high-quality maize to aid in increasing the gains for growth and lactation of ruminants (Jung and Allen, 1995).

The University of Wisconsin Maize Breeding Program initiated an S_1 family recurrent selection project in 1985 to develop experimental germplasm with altered fiber composition. Buendgen et al. (1990) first described the WFISIHI and WFISILO maize populations that were developed to be high and low, respectively, for NDF, lignin, and silica concentrations of the leaf sheath. These populations were used initially to evaluate the relationship of fiber composition with European corn borer [*Ostinia*

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Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; *bm3*, *brown-midrib3*; CP, crude protein; DM, dry matter; ECB, European corn borer; IVTD, in vitro true digestibility; L, lignin; NDF, neutral detergent fiber; NDFD, neutral detergent fiber digestibility; NIRS, near-infrared reflectance spectroscopy; SEC, standard error of calibration; SECV, standard error of cross-validation; WQS, Wisconsin Quality Synthetic; WFISIHI, Wisconsin-fiber-silica-high; WFISILO, Wisconsin-fiber-silica-low.

nubilalis (Hübner)] (ECB) resistance. Wolf et al. (1993a, 1993b) then reevaluated the fiber composition and agronomic attributes of these populations, but they also included population topcrosses to adapted inbred lines as a first step toward evaluating silage potential. Wolf et al. (1993b) concluded that WFISIHI generally had both higher concentrations of the cell wall constituents, NDF, acid detergent fiber (ADF), and lignin, as well as lower digestibility. To improve whole-plant digestibility, they suggested that focus should be placed on stover digestibility (Wolf et al., 1993a). Wolf et al. (1993b) concluded that whole-plant nutritional quality could be increased by reducing stover NDF and increasing stover NDFD without sacrificing grain or whole-plant yield.

Beeghly et al. (1997) continued selection in WFISIHI and WFISILO and also evaluated 100 S_1 families from the WFISILO and WFISIHI populations for ECB resistance. They concluded that cell wall components play a minor role in second-generation ECB resistance, but nonetheless, selection for ECB resistance is likely to lower the digestibility and intake of maize silage by ruminants (Beeghly et al., 1997).

Ostrander and Coors (1997) evaluated the compositional changes accompanying divergent selection for cell wall constituents in WFISIHI, and WFISILO after two cycles of S_1 selection. They found that WFISILO and WFISIHI continued to diverge for all compositional traits, with the exception of whorl ADF, and that WFISILO C2 was significantly more susceptible to first- and second-generation ECB than WFISIHI C2.

As a result of these early studies, a recurrent selection project was initiated to increase forage yield and quality of silage maize. A new breeding population was formed from the cross of WFISILO C3 with two high-quality inbred lines, Mo17 and H99, and the resulting population was named the WQS. WQS was designed to function as a breeding population for the development of inbred lines, that when crossed to inbred lines from the Stiff Stalk heterotic background, would produce hybrids with high forage yield and excellent nutritional quality. The focus of selection, therefore, changed from S_1 per se selection for stover characteristics at anthesis, which was used for the development of WFISIHI and WFISILO, to selection based on S_2–topcross selection for whole-plant yield and quality at the time of silage harvest. Two cycles of selection have been completed for WQS.

The objectives of the present study are to evaluate the forage yield and quality of the WFISIHI, WFISILO, and WQS germplasm over cycles of selection and to determine whether it is feasible to select for both high whole-plant yield and nutritional quality.

MATERIALS AND METHODS

Germplasm

The WFISIHI, WFISILO, and WQS maize populations were included for evaluation. The procedures used to create WFISIHI and WFISILO and subsequent cycles of S_1 selection are described elsewhere (Buendgen et al., 1990; Wolf et al., 1993a, 1993b; Beeghly et al., 1997; Ostrander and Coors, 1997). In brief, the initial populations WFISIHI C0 and WFISILO C0 were distinct from one another, they were broadly based genetically, and they were derived from accessions adapted to temperate conditions from several regions in the world (Buendgen et al., 1990). The initial screening of accessions involved selection for high and low concentrations of ADF, lignin, and silica in the leaf sheath at anthesis to form WFISIHI C0 and WFISILO C0, respectively. The C0 populations were then selected by evaluating 100 S_1 families each cycle for either NDF or ADF and lignin. Ten families with the highest or lowest values were intermated to create the next cycle of WFISIHI or WFSILO, respectively.

WQS was developed following the completion of the third cycle of selection for WFISILO. The 10 selected S_1 families that were intermated to form WFISILO C3 were crossed to the single cross Mo17 × H99, and all three-way crosses were intermated for two generations to create the initial cycle (C0) of WQS. Inbreds Mo17 and H99 were chosen on the basis of their low concentrations of NDF, ADF, and lignin, as well as on their high IVTD and NDFD (Coors and Lauer, 2001). In addition, because Mo17 and H99 represent the non-Stiff Stalk heterotic background, their incorporation into WQS established the combining ability pattern of the WQS population.

The S_2 topcross recurrent selection program was initiated in 1994 by selfing approximately 200 plants in WQS C0. The following year the resulting S_1 families were visually evaluated for plant health and general agronomic acceptability in a field trial at Madison, WI, with three replications planted at a density of about 74 000 plants ha^{-1}. Approximately one-half were discarded, and the remaining 110 S_2 families were topcrossed to inbred LH119. These topcrosses were evaluated at two locations, Madison and Arlington, WI, for whole-plant yield and nutritional quality. Twenty superior S_2 families were selected on the basis of a heritability-based selection index consisting of total whole-plant yield, NDF, IVTD, NDFD, and CP. The selected S_2 families were recombined by the bulk entry method to form WQS C1. The second cycle of topcross selection began in 1999 when 365 S_1 families were developed from WQS C1 and visually screened at Madison, WI, in a field trial with three replications planted at a density of about 74 000 plants ha^{-1}. Ninety-two S_2 families derived from selected S_1 families were topcrossed to inbred LH198, and these testcrosses were evaluated in 2000 for whole-plant yield, NDF, IVTD, NDFD, CP, and starch. In contrast to WQS C1, the selection criterion was based on milk yield per hectare estimated by MILK2000 predictions as developed by Schwab et al. (2003). The milk per hectare values represent a selection index combining whole-plant yield and predicted milk production based on NDF, NDFD, CP, and starch concentration. Twenty S_2 families from WQS C1 were selected on the basis of superior yield and milk production potential based on MILK 2000 predictions, and they were recombined by the bulk entry method to create WQS C2. Population WQS C2 has been released by the University of Wisconsin (Coors, 2003).

Included in this study were cycles 0, 1, 2, and 3 of the WFISIHI and WFISILO populations, and cycles 0, 1, and 2 of WQS. WQS C2 was only included in the second year of the study because it had not yet been developed by the first year of the study.

Population topcrosses of WQS C0 and C1 with inbred lines LH119, LH198, and LH200 were also included in the study. Each population cross was made with at least 50 plants from each cycle of WQS crossed to each inbred line. Equal quantities of seed from each topcrossed plant were composited to form the population topcross. The cross Mo17 × H99 was included in the trial because Mo17 and H99 were involved in the development of the WQS population. The check entries included 71712-B-1-1-3-1-B × LH119, 71712-B-1-1-3-1-B × LH198, and three commercial hybrids Pioneer brand P33A14 (Pioneer Hi-Bred Intl., Johnston, IA), F657 (Mycogen, Indianapolis, IN), and TMF113 (Mycogen, Indianapolis, IN). Inbred line 71712-B-1-1-3-1-B was an advanced (S_6+) line related to a series of "lax leaf" families developed from WFISILO C0 that were selected for high stover digestibility and low concentrations of stover NDF and lignin (Falkner et al., 2000). Inbred line 71712-B-1-1-3-1-B will be designated hereafter as "71712". Hybrid P33A14 represented a high-yielding grain hybrid. Brown-midrib hybrid F657, which carries the *bm3* allele reducing lignin concentration, served as a high-quality check. Hybrid TMF113 has the leafy characteristic, which increases the leaf number above the ear typically increasing whole-plant yield, and, therefore, TMF113 served as a high-biomass check.

Field Evaluation

All germplasm was grown and evaluated for stover and whole-plant composition in 2000 and 2001 with the exception of the second cycle of WQS, which was only evaluated in 2001. The experiment was conducted at two locations, Madison and Arlington, WI. Soil type at both locations is Plano silt loam (fine-silty, mixed mesic Typic Arguidoll). The experiment was planted in a randomized complete block design with three replicates. Plots consisted of two rows 6.1 m long and 0.76 m apart planted at approximately 79 000 plants ha^{-1}. The plots were planted on 3 May 2000 and 2 May 2001 at Madison, WI, and 22 May 2000 and 18 May 2001 at Arlington, WI. All trials were fertilized according to soil recommendation. Weeds were controlled by application of cyanazine [1.7 kg ha^{-1} a.i.; 2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropionitrile] and alachlor (0.9 kg ha^{-1} a.i.; 2-chloro-2′,6′-diethyl-*N*-methoxymethylacetanilide) before plants emerged, and plots were also weeded by hand throughout the season. In 2000, there were two separate harvest dates per location (8, 16 September at Madison and 21, 27 September at Arlington), and in 2001 there were three separate harvest dates per location (6, 13, 18 September at Madison and 25 September, 1, 5 October at Arlington). The entries harvested on a specific harvest date were determined on the basis of flowering times. The separate harvests were used so the germplasm would be at a common physiological stage when harvested. Days to mid-silk and days to mid-pollen were measured as the days from planting until silks or shedding tassels, respectively, were evident on 50% of the plants in each plot. Ten plants within each plot were measured for plant and ear height shortly after pollination. Plant heights were measured as the distance from the ground to the collar of the uppermost leaf, and ear heights were measured as the distance from the ground to the uppermost ear node. At harvest, approximately 300 to 400 g kg^{-1} DM, one row of each two-row plot, was stripped of ears (for stover analysis), and each row was either manually harvested by cutting the stalk at 16 cm above the ground (harvest one in 2000 and harvests one and two in 2001) then fed through a one-row, tractor-mounted forage chopper (New Holland 707, New Holland, PA) or mechanically harvested with the same forage chopper (harvest two in 2000 and harvest three in 2001). Each row was weighed, and a 1-kg sample was collected for moisture and quality measurements. The forage sample was dried at 55°C for 1 wk, and wet and dry weights were used to calculate DM concentrations. Yield was calculated on a 100% DM basis. The difference between stover and whole-plant weight was divided by whole-plant weight to determine ear percentage. Dried samples were ground to pass through a 1-mm screen using a Christy hammer mill (Christy, Suffolk, UK).

Laboratory Procedures

All the samples were scanned with a NIRSystems 6500 near infrared reflectance spectrophotometer (NIRS) (FOSS NIRSystems Inc., Silverspring, MD). Standard NIRS procedures were used (Martens and Naes, 1989; Shenk and Westerhaus, 1991, 1994). The CENTER program was used to compute standardized *H* (Mahalanobis) statistics for each sample's spectra, and the SELECT program was used to select samples for wet-lab analysis using a standardized *H* of 1.0 for both whole-plant and stover materials (Shenk and Westerhaus, 1991).

Selected whole-plant and stover samples were then analyzed for fiber composition and digestibility. A modified procedure of Goering and Van Soest (1970) was used for sequential analysis of NDF, ADF, and acid detergent lignin (ADL). Modifications included use of an ANKOM^{200} fiber analyzer (Ankom Technologies Corp., Fairport, NY); 0.5-g samples were placed in Ankom F57 filter bags and were closed by heat-sealing for NDF and ADF determinations; addition of 5% (v/v) heat-stable α-amylase in the NDF procedure during refluxing and again during the rinsing stage; and elimination of decalin. Total nitrogen content was analyzed by means of a 0.12-g sample that was combusted in a Leco FP-528 Nitrogen Analyzer (LECO Corporation, St. Joseph, MI). Crude protein was calculated by multiplying total nitrogen by 6.25.

Duplicate 0.5-g samples were used to determine IVTD by a modification of the procedure used by Darby and Lauer (2002). The DAISY II unit was post-fabricated with a CO_2 system to supply constant CO_2 throughout the experiment. Following incubation, the samples were rinsed with water and then frozen until NDF analysis was conducted by the modified procedure described earlier.

The NIRS calibration sets for 2000 and 2001 stover were combined to provide a single calibration set. Similarly, the 2000 and 2001 whole-plant calibration sets were combined. With data from the wet-lab analysis, prediction equations were developed relating the NIRS spectra to each quality variable NDF, ADF, ADL, IVTD, and CP (Shenk and Westerhaus, 1991; 1994). Selection of prediction equations was based on high R^2 values and low standard errors of calibration (SEC) and cross-validation (SECV) (Martens and Naes, 1989). To evaluate the accuracy of ADL estimates for the most critical subset of entries, whole-plant and stover samples of WQS C2, F657, and P33A14 collected from each replication at Madison and Arlington in 2001 were analyzed by the ADL wet-laboratory procedures mentioned previously.

Starch was predicted with a global NIRS equation developed by the University of Wisconsin Silage Breeding and Maize Extension Programs in the Department of Agronomy (Coors, 2003). The lignin/NDF ratio (L NDF^{-1}) was calculated as ADL divided by NDF. NDFD was calculated from predicted NDF and IVTD values by the equation: $\mathrm{NDFD} = 100\{[\mathrm{NDF} - (100 - \mathrm{IVTD})]/\mathrm{NDF}\}$. Milk yield per megagram DM was estimated by MILK2000, developed by Schwab et al. (2003); and milk per hectare was estimated for each plot by multiplying milk per megageam by whole-plant yield.

Statistical Procedures

Analyses of variance were calculated by the general linear model approach. Data from each environment (year–location combination) were analyzed according to the randomized complete block design. For the traits involving DM yields, i.e., whole-plant yield, stover yield, and milk per hectare, the number of plants in each plot was used as a covariate within each environment. Least squares means for each environment were used for analyses over environments. Environments and replications were considered random effects, and entries were considered fixed effects. Data are summarized herein as least squares means for each entry and trait over environments. Means for WQS C2 are adjusted means estimated from only the two locations in 2001. Regression analysis was used to determine whether there were significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$) linear trends in composition or agronomic performance over cycles of selection in WFISIHI and WFISILO.

RESULTS AND DISCUSSION

NIRS Prediction

Nearly all compositional and quality traits were well predicted by NIRS (Table 1). With few exceptions, R^2 exceeded 0.90 and SECs and SECVs were less than 5% of the trait mean. The exceptions involved whole-plant and stover lignin and stover IVTD. The R^2 for stover IVTD was 0.83, which is low, but not exceptionally so for this trait. The whole-plant lignin R^2 was 0.91, but the SEC and SECV values exceeded 10% of the trait mean. The stover lignin R^2 was 0.59, and the SEC and SECV values again exceeded 10% of the trait mean. Lignin concentration is typically one of the more difficult components to measure because of its relatively low concentration and the gravimetric procedures used to estimate it.

Table 1. Statistics of near infrared reflectance spectroscopy (NIRS) calibration and prediction for maize whole-plant and stover quality traits.

	NIRS statistics					
Trait†	Equation	Mean	N‡	R^2	SEC§	SECV¶
			Whole plant			
NDF, g kg^{-1}	1,2,2,1	545	93	0.94	21	25
ADF, g kg^{-1}	1,2,2,1	274	91	0.96	12	14
IVTD, g kg^{-1}	1,2,2,1	663	91	0.91	19	21
ADL, g kg^{-1}	1,2,2,1	38	91	0.91	4	6
CP, g kg^{-1}	1,2,2,1	84	94	0.95	3	4
			Stover			
NDF, g kg^{-1}	1,4,4,1	676	82	0.95	14	16
ADF, g kg^{-1}	1,4,4,1	365	82	0.95	10	11
IVTD, g kg^{-1}	2,4,4,1	581	84	0.83	20	26
ADL, g kg^{-1}	2,7,7,1	53	79	0.59	8	9
CP, g kg^{-1}	2,4,4,1	75	83	0.94	3	5

† DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; IVTD, in vitro true digestibility; ADL, acid detergent lignin; CP, crude protein.

‡ N corresponds to the final number of data points used in the NIRS calibration equation.

§ SEC = standard error of calibration.

¶ SECV = standard error of cross-validation.

Influence of Selection on Whole-Plant Nutritional Quality

WFISIHI and WFISILO Evaluation

Divergent selection for fiber concentrations for the WFISIHI and WFISILO populations resulted in differences for NDF, ADF, IVTD, ADL, L NDF^{-1}, NDFD, starch, and milk yield Mg^{-1} DM (Table 2). In general,

Table 2. Whole-plant forage quality evaluation of the WFISIHI, WFISILO, and WQS maize populations over cycles of selection. Data were obtained from replicated field trials at Madison and Arlington, WI, in 2002 and 2001.

Entry or trait†	NDF	ADF	IVTD	ADL	L NDF^{-1}	CP	NDFD	Starch	Milk yield‡	Milk yield§
	g kg^{-1}								kg milk Mg^{-1} DM	kg milk ha^{-1}
WFISIHI C0	557	280	634	43	76	85	342	197	1091	12 273
WFISIHI C1	543	264	638	40	73	87	335	214	1089	11 022
WFISIHI C2	588	298	604	49	82	92	327	152	979	8 789
WFISIHI C3	584	297	614	48	81	91	338	172	1013	10 175
WFISILO C0	560	283	648	41	72	84	373	167	1140	12 244
WFISILO C1	545	269	666	38	69	88	386	194	1212	9 868
WFISILO C2	540	268	665	35	64	86	378	215	1200	9 392
WFISILO C3	526	262	670	32	61	90	372	214	1192	7 710
WQS C0	532	267	676	36	68	84	392	201	1239	10 533
WQS C1	525	258	685	33	62	85	398	221	1270	9 685
WQS C2¶	504	242	703	31	61	84	413	225	1337	14 173
WQS C0 × LH119	538	267	667	39	73	78	380	228	1222	19 078
WQS C0 × LH198	529	267	672	37	70	78	381	221	1228	19 043
WQS C0 × LH200	543	278	659	41	76	84	372	207	1192	17 174
WQS C0 testcross mean	536	271	666	39	73	80	378	218	1214	18 432
WQS C1 × LH119	519	256	674	35	67	80	370	248	1240	20 033
WQS C1 × LH198	534	266	667	36	68	79	376	239	1216	20 339
WQS C1 × LH200	533	271	663	39	72	82	368	232	1208	17 625
WQS C1 testcross mean	528	264	668	37	69	81	372	240	1221	19 332
Mo17 × H99	506	247	700	38	74	80	409	249	1322	16 669
71712 × LH119	513	249	686	39	76	79	389	253	1265	20 085
71712 × LH198	511	251	689	34	65	74	391	250	1291	19 546
F657 (brown midrib)	509	248	725	30	58	83	460	266	1414	17 513
P33A14	522	263	679	36	68	78	386	252	1262	23 755
TMF113 (leafy)	587	307	614	47	81	85	342	156	1027	17 626
Trial mean	537	268	665	38	70	83	377	216	1202	14 972
LSD 1 (0.05)#	26	19	22	6	9	6	23	37	88	4 110
LSD 2 (0.05)††	32	24	27	7	11	8	28	45	108	5 033
LSD 3 (0.05)‡‡	15	11	13	3	5	4	13	21	51	2 373

† NDF, neutral detergent fiber; ADF, acid detergent fiber; IVTD, in vitro true digestibility; ADL, acid detergent lignin; L NDF^{-1}, lignin-NDF ratio; CP, crude protein; NDFD, NDF digestibility.

‡ MILK2000 estimate of kg of milk production Mg^{-1} dry matter (DM) of forage.

§ MILK2000 estimate of kg of milk production ha^{-1} of forage.

¶ WQS C2 was only evaluated in 2001 at Madison and Arlington, WI.

Least significant difference for all comparisons except those involving WQS C2 and those of WQS C0 and WQS C1 testcross means.

†† Least significant difference for all comparisons involving WQS C2.

‡‡ Least significant difference for comparisons of WQS C0 and WQS C1 testcross means.

when differences did exist, they were usually between WFISIHI C3 and WFISILO C3, with WFISIHI being of lower quality than WFISILO. For WFISILO there were significant linear decreases in whole-plant NDF, and highly significant linear decreases for ADL and L NDF^{-1} ($b_{\text{lin}} = -11, -3, -4$ g kg^{-1} cycle^{-1}, respectively) over cycles of selection. Milk yield acre^{-1} also had a significant linear decrease for WFISILO ($b_{\text{lin}} = -1408$ kg milk ha^{-1} cycle^{-1}), which was mostly related to a decrease in agronomic productivity (see below).

Values for NDF and ADF of the WFISIHI C0 and WFISILO C0 were similar to those reported by Wolf et al. (1993b), but the IVTD and NDFD values of the present study were lower than those reported by Wolf et al. (1993b) (Table 2). The differences in IVTD and NDFD are most likely due to different analytical techniques. Wolf et al. (1993b) used the technique described by Marten and Barnes (1980) to determine IVTD, while the present study used the DAISY II system and the Ankom F57 filter bags. In addition, Wolf et al. (1993b) used 0.25-g samples for analysis whereas 0.5-g samples were used in the present study. Wilman and Adesogan (2000) found that the filter bag method using Ankom F57 filter bags and 0.5-g samples underestimated the IVTD and NDFD when compared with the traditional Tilley and Terry (1963) technique.

All cycles of WFISIHI had greater NDF concentrations than F657, and the same was true for ADF with the exception of WFISIHI C1 (Table 2). WFISILO C3 had concentrations of NDF and ADF similar to F657. WFISILO C2 and C3 had relatively low ADL concentrations that were equivalent (i.e., not significantly different based on LSD values) to F657, whereas all cycles of WFISIHI had greater ADL than F657. WFISILO C2 and C3 were lower than WFISIHI C2 and C3 for L NDF^{-1}. All cycles of WFISIHI had greater L NDF^{-1} than F657, whereas L NDF^{-1} of WFISILO C2 and C3 were equivalent to F657. All cycles of WFISIHI and WFISILO populations had lower IVTD, NDFD, and predicted milk per megagram yield DM than F657. All cycles of WFISIHI and WFISILO yielded less milk per hectare than P33A14, the entry with the highest milk yield ha^{-1}.

WQS Per Se Evaluation

There was no significant change in nutritional composition between WQS C0 and C1, but WQS C2 showed several improvements in quality (Table 2). In general, two cycles of selection in WQS either improved whole-plant quality above that of its progenitor population, WFISILO C3 (i.e., IVTD, NDFD, milk Mg^{-1} DM, milk ha^{-1}), or had no significant effect. The only exception was CP, which was lower for WQS C2 than WFISILO C3. The ADL concentration and the L NDF^{-1} ratio of WQS C2 were the second lowest values for these traits, and they were equivalent to the brown-midrib hybrid F657. As a likely result, milk yield per megagram DM for WQS C2 was also the second highest in the trial, and again equivalent to F657.

The combination of Mo17 and H99 with WFISILO C3 to create WQS C0 appears to have been a positive step toward increased nutritional quality. The hybrid Mo17 × H99 had the lowest whole-plant NDF and ADF concentrations of all the entries, and it had a whole-plant IVTD equal to F657 (Table 2). The whole-plant quality of WQS C0 was equivalent to that of either of its progenitors, WFISILO C3 and Mo17 × H99.

Testcross Evaluation

As expected, testcrossing cycles of WQS tended to reduce nutritional quality below that of respective cycles per se because the tester inbreds were not selected on the basis of quality, but rather for hybrid yield potential (Table 2). The testcross changes from C0 to C1 mostly followed the same pattern for per se changes from C0 to C1, but all changes, with the exception of starch, were not significant. In general, the whole-plant nutritive value of the WQS C1 testcrosses was equivalent to the commercial hybrid check P33A14. Most of the WQS testcrosses had lower ADL and higher milk yield per megagram DM than TMF113. All WQS testcrosses, P33A14, and TMF113 had lower NDFD and milk yield per megagram than the brown-midrib check F657.

The whole-plant quality of the 71712 testcrosses with LH119 and LH198 showed that relatively high-quality hybrids (i.e., hybrids with low fiber, high digestibility, and high milk yield Mg^{-1} DM) could be created with inbreds from the WFISILO population, but overall the 71712 testcrosses were not nutritionally superior to the commercial hybrid check with the highest milk yield per hectare, P33A14.

Influence of Selection on Stover Nutritional Quality

WFISIHI and WFISILO Evaluation

While the direction of changes in stover quality over cycles of selection for WFISIHI and WFISLO were mostly in the expected directions, the magnitudes of the changes were not significant in most instances. Nonetheless, by the third cycle, WFISIHI and WFISILO had diverged significantly for stover NDF, ADF, IVTD, ADL, and NDFD (Table 3). The results of this study differ somewhat from those reported by Ostrander and Coors (1997) for stover composition. Ostrander and Coors (1997) observed significant decreases in stalk, leaf sheath, and blade tissues for NDF and ADF concentrations for WFISILO over C0, C1, and C2, whereas the present study did not find significant changes in stover NDF and ADF in WFISILO from C0 to C3. This is probably due to a number of factors, most obvious being the difference in tissue used for analysis. Ostrander and Coors (1997) separated the stover into different fractions, and the present study used the entire stover fraction. Further, Ostrander and Coors (1997) harvested their material at anthesis, 65 to 75 d after planting (which was the stage when selection occurred), while our material was harvested at the stage most appropriate for silage harvest, 300 to 400 g kg^{-1} DM. Ostrander and Coors (1997) harvested material before pollination when more nonstructural carbohydrate would be present in the stover fraction of the plant, which may have created more variation in quality traits than would be present after

Table 3. Stover quality evaluation of the WFISIHI, WFISILO, and WQS maize populations over cycles of selection. Data were obtained from replicated field trials at Madison and Arlington, WI, in 2002 and 2001.

Entry or Trait†	NDF	ADF	IVTD	ADL	L NDF^{-1}	CP	NDFD
	g kg^{-1}						
WFISIHI C0	711	389	550	60	84	72	367
WFISIHI C1	706	379	551	60	85	79	364
WFISIHI C2	723	392	518	63	87	82	333
WFISIHI C3	727	395	513	62	85	82	330
WFISILO C0	662	355	587	51	77	75	376
WFISILO C1	671	360	599	51	75	74	403
WFISILO C2	673	362	584	53	78	81	380
WFISILO C3	676	362	587	55	80	84	388
WQS C0	646	342	611	49	75	79	398
WQS C1	662	354	611	50	74	75	413
WQS C2‡	644	338	617	45	69	73	405
WQS C0 × LH119	685	372	568	56	81	71	369
WQS C0 × LH198	685	369	582	52	76	67	389
WQS C0 × LH200	686	376	566	56	81	74	367
WQS C0 testcross mean	685	372	572	54	79	71	375
WQS C1 × LH119	687	373	572	56	81	71	378
WQS C1 × LH198	706	389	566	57	81	73	385
WQS C1 × LH200	690	378	569	56	81	73	375
WQS C1 testcross mean	694	380	569	56	81	72	379
Mo17 × H99	670	357	597	51	76	71	399
71712 × LH119	700	380	572	56	79	73	388
71712 × LH198	694	384	597	52	74	66	419
F657 (brown midrib)	717	391	608	53	74	77	452
P33A14	721	398	533	58	80	77	352
TMF113 (leafy)	706	382	528	57	80	81	329
Trial mean	689	373	573	55	79	75	381
LSD 1 (0.05)§	24	18	26	5	6	8	25
LSD 2 (0.05)¶	30	22	31	6	7	9	30
LSD 3 (0.05)#	14	10	15	3	3	4	14

† NDF, neutral detergent fiber; ADF, acid detergent fiber; IVTD, in vitro true digestibility; ADL, acid detergent lignin; L NDF^{-1}, lignin-NDF ratio; CP, crude protein; NDFD, NDF digestibility.

‡ WQS C2 was only evaluated in 2001 in Madison and Arlington, WI.

§ Least significant difference for all comparisons except those involving WQS C2 and those of WQS C0 and WQS C1 testcross means.

¶ Least significant difference for all comparisons involving WQS C2.

Least significant difference for comparisons of WQS C0 and WQS C1 testcross means.

grain fill. Coors et al. (1997) reported that, with the exception of whole-plant CP and stover NDFD, whole-plant quality increased and stover quality decreased as ear-fill increased. Albrecht et al. (1986) and Coors et al. (1997) theorized that this inverse relationship between ear fill and nutritional quality is due to remobilization of nonstructural carbohydrates from the plant to the ear. Finally, the planting density was much higher in the current study (79 000 plants ha^{-1}) than that used by Ostrander and Coors (1997) (54 000 plants ha^{-1}). Increased plant density has been found to reduce plant nutritional quality (Cox and Cherney, 2001).

WQS Per Se Evaluation

As with whole-plant quality, two cycles of selection in WQS C0 either improved stover quality above that of its progenitor population, WFISILO C3 (i.e., NDF, ADF, ADL, L NDF^{-1}), or had no significant effect (Table 3). Again, the only exception was CP, which was lower for WQS C2 than WFISILO C3. The ADL concentrations of WQS C0, C1, and C2 were the three lowest values recorded for all entries. WQS C2 had the lowest NDF, ADF, and ADL in the study, and its IVTD was the highest in the study. The stover quality of WQS C2 was superior to the brown-midrib check, F657, for NDF, ADF, and ADL and equivalent for IVTD, L NDF^{-1}, and CP.

The wet-lab analysis of ADL for WQS C2, F657, and P33A14 confirms the trends in ADL concentration estimated by use of NIRS, with two exceptions (Table 4). On the basis of the NIRS estimates (Table 2), WQS C2 whole-plant ADL did not significantly differ from P33A14, but the 5 g kg^{-1} difference between WQS C2 and P33A14 was significant for the wet-lab analysis of 2001 samples (Table 4). On the other hand, while stover ADL of WQS C2 was significantly lower than that of F657 using NIRS estimation (Table 3), the difference was not statistically significant using wet-lab analysis (Table 4).

As was evident for whole-plant quality, Mo17 and H99 appear to have been beneficial contributions to the formation of WQS. Mo17 × H99 had lower stover NDF and ADF, and its stover IVTD, ADL, and L NDF^{-1} were similar to F657 (Table 4).

Testcross Evaluation

The WQS C0 and C1 testcross means for stover NDF, ADF, and ADL were as low as any check entry. The NDFD of the WQS C1 testcross mean was higher than

Table 4. Acid detergent lignin (ADL) determined from wet-laboratory analysis for maize population WQS C2, and hybrids F657 and P33A14 in 2001 at Madison and Arlington, WI.

Entry	Whole-plant ADL	Stover ADL
	g kg^{-1}	
WQS C2	27	39
F657 (brown midrib)	27	45
P33A14	32	56
Mean	29	47
LSD (0.05)	4	10

that for TMF113 and P33A14. However, the brown-midrib check, F657, had higher NDFD than all other entries in the trial.

The stover quality of the 71712 testcrosses with LH119 and LH198 again confirms that relatively high-quality hybrids could be produced using inbreds from the WFISILO population. In particular, 71712 × LH198 had higher IVTD and NDFD than both P33A14 and TMF113, and its IVTD did not differ from the brown-midrib check, F657.

Influence of Selection on Agronomic Attributes

The entries in this study were grouped according to maturity, and those with different maturity groupings were harvested on separate dates to ensure that all entries were harvested at a comparable physiological stage. Nonetheless, there were significant differences among entries for whole-plant and stover DM. However, all entries were within a range of 311 to 400 g kg^{-1} whole-plant DM, which is a relatively narrow range appropriate for comparing silage varieties (Table 5).

WFISIHI and WFISILO Evaluation

Stover yield of WFISIHI and both whole-plant and stover yield of WFISILO decreased over cycles of selection (Table 5). By the third cycle, WFISIHI C3 had greater whole-plant and stover yields than WFISILO C3, and the two populations had diverged for plant height, ear height, days to mid-silk, and days to mid-pollen, mostly because of changes in WFISILO. Over cycles of selection, WFISILO tended to flower earlier, have shorter stature, and have lower yield. There were significant linear decreases in plant and ear height ($b_{lin} = -14, -10$ cm cycle^{-1}, respectively). Even though whole-plant and stover yield of WFISILO C3 was markedly lower than WFISILO C0, there was a highly significant linear increase in ear percentage over cycles of selection for WFISILO (b_{lin} = 6% cycle^{-1}).

WQS Per Se Evaluation

WQS C0 and C1 did not differ for whole-plant yield, but whole-plant yield of WQS C2 was significantly greater than WQS C1. Plant and ear heights did not change from C0 to C1 for the WQS population, although ear heights of WQS C2 were higher than WQS C0. Flowering dates of WQS C0 and C1 did not differ from each other and were intermediate to the WQS progenitors WFISILO C3 and Mo17 × H99. Flowering dates for WQS C2 were later than WQS C0 and WFISILO C3, and it seems possible that later flowering may have been an indirect effect of the selection protocol.

The Milk2000 prediction of milk yield involves a DM adjustment of starch digestibility because, even though starch concentration increases as plants mature, starch becomes harder to degrade in the rumen as kernels approach black-layer formation. The Milk2000 adjustment decreases starch digestibility for unprocessed silage by 16.7 g kg^{-1} for every 10 g kg^{-1} increase in DM

Table 5. Evaluation of agronomic performance for the WFISIHI, WFISILO, and WQS maize populations over cycles of selection. Mid-silk and mid-pollen dates were recorded at one location, Madison, WI, in 2000 and 2001. All other traits were recorded at Arlington and Madison in both years.

Entry	Whole-plant dry matter	Stover dry matter	Whole-plant yield	Stover yield	Ear percent	Plant height	Ear height	Mid-silk	Mid-pollen
	g kg^{-1}		Mg ha^{-1}			cm		d	
WFISIHI C0	369	311	11.2	6.7	35	201	102	82	80
WFISIHI C1	400	340	9.9	5.4	39	184	90	82	80
WFISIHI C2	352	320	9.0	5.8	36	192	95	83	80
WFISIHI C3	382	308	10.0	5.2	49	194	98	85	81
WFISILO C0	316	268	10.5	6.8	32	197	106	82	79
WFISILO C1	345	286	8.0	4.2	40	176	89	79	77
WFISILO C2	374	313	7.7	4.0	47	167	84	79	77
WFISILO C3	398	329	6.4	3.2	49	153	75	78	76
WQS C0	339	276	8.3	4.1	46	161	74	80	77
WQS C1	348	272	7.2	3.9	50	166	78	80	78
WQS C2†	315	242	10.6	5.5	44	170	84	82	80
WQS C0 × LH119	352	263	15.5	7.6	51	205	96	82	81
WQS C0 × LH198	344	278	15.5	8.4	46	210	105	83	81
WQS C0 × LH200	312	254	14.3	8.0	43	210	108	85	83
WQS C0 testcross mean	336	265	15.1	8.0	47	209	103	83	82
WQS C1 × LH119	352	278	16.0	7.8	50	208	104	83	80
WQS C1 × LH198	359	292	16.7	8.1	51	208	105	83	81
WQS C1 × LH200	318	259	14.6	7.7	46	210	111	84	83
WQS C1 testcross mean	343	276	15.7	7.9	49	208	107	83	81
Mo17 × H99	339	280	12.6	6.5	50	191	84	83	81
71712 × LH119	385	295	15.9	7.4	54	220	103	82	79
71712 × LH198	363	266	15.1	6.8	53	222	111	82	79
F657 (brown midrib)	338	272	12.7	6.8	42	199	100	84	83
P33A14	334	264	18.9	9.1	50	226	124	85	84
TMF113 (leafy)	311	278	16.7	11.4	26	264	111	90	91
Trial mean	350	284	12.3	6.5	45	197	97	82	80
LSD 1 (0.05)‡	39	29	3.1	1.3	13	8	7	2	2
LSD 2 (0.05)§	48	35	3.8	1.6	16	10	9	2	3
LSD 3 (0.05)¶	23	16	1.8	0.8	8	5	4	1	1

† WQS C2 was only evaluated in 2001 at Madison and Arlington, WI.

‡ Least significant difference for all comparisons except those involving WQS C2 and those of WQS C0 and WQS C1 testcross means.

§ Least significant difference for all comparisons involving WQS C2.

¶ Least significant difference for comparisons of WQS C0 and WQS C1 testcross means.

(Schwab et al., 2003). Therefore, using MILK2000 as a selection index for nutritive value in a silage-breeding program may lead to an unintended delay in flowering. This may be one reason why WQS C2 flowered later than earlier cycles. Greater attention to maturity during future cycles of selection is needed to ensure that WQS remains adapted to its intended target area, the Northern Corn Belt.

Testcross Evaluation

As expected, there was a significant and dramatic increase in yield when either WQS C0 or C1 was crossed to the inbred testers (LH119, LH198, or LH200). Whole-plant yields of the WQS C1 testcrosses were equivalent to the highest-yielding hybrid checks, P33A14 and TMF113, with the exception that WQS C1 × LH200 yielded less than P33A14. Stover yields of the WQS testcrosses were comparable to most of the check hybrids; however, no testcross had stover yields equivalent to the highest-yielding leafy hybrid, TMF113.

Plant heights of the WQS testcrosses were lower than the hybrid checks in most instances, while ear heights of the WQS testcrosses were mostly comparable to the hybrid checks. The notable exceptions involved the leafy hybrid TMF113, for which the plant height exceeded all other entries, and P33A14, for which ear height exceeded all other entries. The flowering dates of WQS testcrosses tended to be later than those of the population per se. This was expected because the inbred testers LH119, LH198, and LH200 flowered 1 to 4 d later than the WQS germplasm.

The testcrosses of 71712 with LH119 and LH198 were included to evaluate the potential of a high-quality inbred derived from the initial cycle of WFISILO when crossed to inbreds from the Stiff Stalk heterotic group (Table 5). The whole-plant and stover yields of the 71712 testcrosses were lower, although not significantly so, for whole-plant yield, from the highest yielding check hybrids P33A14 and TMF113. The WQS testcrosses indicated that advanced cycles of WQS might be a good source of inbreds with heterotic potential. The average whole-plant yield of WQS C1 testcrosses was 84% of the highest-yielding hybrid check, P33A14, which is good for testcrosses involving a genetically heterogeneous population (versus testcrosses involving only inbred lines).

Influence of Starch and Protein on Nutritional Quality

The starch fraction may have a prominent role in determining silage quality when hybrid germplasm such as the hybrid checks and population testcrosses are compared with inbred germplasm or populations. This is due to the increase in grain yield produced by heterotic interactions in hybrids or testcrosses. In this light, the favorable comparison of the population WQS C2 with the hybrid F657 is noteworthy. The stover composition of WQS C2 is equivalent in quality to F657, and for several traits it is even superior. As a result, WQS C2 was second only to F657 for milk yield per megagram DM (Table 2) even though WQS was handicapped somewhat by its relatively low starch concentration when compared with most of the hybrid checks. This highlights the need to continue selection for elevated starch concentrations using a selection index such as MILK2000. It also seems reasonable that, to more fully realize the increase in quality in inbred lines derived from WQS C2 and subsequent selection cycles, it might be prudent to develop high-quality inbreds from at least one other genetically distinct and complementary combining ability group.

There has been little improvement for either stover or whole-plant CP in WQS. The reasons for this are somewhat unclear, but there tends to be a negative correlation between starch and protein concentration in maize for both grain and silage. In the current study, the phenotypic correlation of whole-plant starch and protein was $r = -0.71$, which was highly significant. Over decades of breeding for high grain yield in maize, protein concentration has decreased (Duvick and Cassman, 1999), and this may be because the size of the endosperm has increased at the expense of the of the germ portion of the seed, where the greatest portion of grain protein is concentrated. In the WQS selection program, starch may receive more weight than protein because it contributes not only to nutritive value via the MILK2000 selection index, but also to whole-plant yield.

SUMMARY

It is difficult to point to any specific selection criterion used in the WFISIHI, WFISILO, and WQS programs that was most useful in modifying nutritive value. The selection criteria used in the different populations and within each population changed over time. Regardless of their differences, the divergent selection programs involving WFISIHI and WFISILO clearly demonstrate that selection based on stover composition, even at an early stage of development such as anthesis, can create marked changes in both stover and whole-plant composition when measured at a later physiological stage. It also appears from the WQS selection program that selection based on whole-plant quality evaluation of S_2–topcrosses is effective, especially when conducted using selection indices incorporating whole-plant yield.

The combination of WFISILO C3 with inbred lines Mo17 and H99 to create the WQS germplasm provided the opportunity to combine excellent nutritional attributes and establish a productive heterotic pattern for whole-plant yield. There is relatively little genetic variation for silage quality, particularly stover quality, among conventional maize hybrids used for grain in the USA (Coors and Lauer, 2001; Lauer et al., 2001), and the WQS germplasm would appear to have unique potential for silage breeders. What is needed for the future is a hybrid with the stover quality of a brown-midrib such as F657, the stover yield of a leafy hybrid such as TMF113, and the grain yield and starch content of a high-grain yielding hybrid such as P33A14. The WQS breeding program is an attempt to achieve this end, but while the progress to date is promising, much work remains.

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