ences among the maize genotypes. Amylose and granule size also were examined to determine whether these measurements explained the observed differences in DSC values.

MATERIALS AND METHODS

Plant Materials

The inbred lines Oh43 and A632 and the F1 progeny Oh43 × A632 were planted at four dates (Table I) at Ames, IA, in 1991, in a factorial randomized complete-block design with two replicates. Plots consisted of 12-foot rows thinned to 25 plants. Plants were self-pollinated; flowering date was recorded as the number of days to pollination following an arbitrarily selected date (June 30). The initial planting date was May 15, and subsequent plantings were made at approximately one-week intervals (Table I). Later planting dates resulted in a progressive delay in flowering for each of the three genotypes (Table I). Fluctuations in daily high temperatures throughout the 1991 growing season in relation to the four planting dates are shown in Figure 1. Ears were harvested on a single date (October 15), when all entries had reached physiological maturity (presence of black layer). They were dried for 48 hr at 38°C. Two ears per replicate were selected for analysis, from which 100-kernel weights (HKW) were recorded. Only kernels selected from the center one-third portion of the ear were included in the analysis.

Starch Isolation

Starch was isolated from five kernels per ear. Kernels were steeped (48 hr in 0.45% sodium meta-bisulfite at 50°C), degermed by hand, homogenized in a laboratory microblender, filtered to pass a 30-μm sieve, washed with distilled H_2O, and decanted (White et al 1990). Starches were further purified by toluene emulsification, as described by Krueger et al (1987a).

Differential Scanning Calorimetry

A calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) equipped with a thermal analysis data station was used. Approximately 3.5 mg (dwb) of starch was weighed into an aluminum pan and 8 mg of distilled water was added. The pan was sealed, allowed to equilibrate for approximately 1 hr, and heated from 30 to 102°C at a rate of 10°C/min (White et al 1990). T_o, T_p, and ΔH were recorded directly from the computer software. The R_n of gelatinization was calculated as $2(T_p - T_o)$, according to Krueger et al (1987a). Values for each replicate represented the mean of four DSC runs, averaged from two five-kernel samples per ear and two ears per replicate.

Apparent Amylose

A rapid method was used to determine %AM from starches as described by Knutson (1986). This procedure is as accurate and sensitive as conventional colorimetric methods (Knutson 1986). Approximately 5.0 mg of starch was dissolved in 10 ml of 90% dimethyl sulfoxide containing $6 \times 10^{-3} M$ iodine. Dissolved sample (1 mg) was diluted to 9 ml with H_2O, and the absorbance was measured at 600 nm on a spectrophotometer (Hitachi U-2000, Tokyo, Japan). Purified amylose was prepared from maize starch as described by Schoch (1942), and used to construct a standard curve.

Starch Granule Size

Isolated starch granules were dispersed in ethanol and mounted on slides. The preparations were placed on a Laborlux light microscope fitted with a video camera. The microscope system was attached to a digitizing Colorado video unit (Boulder, CO) linked to a Kevex Delta IV instrument with an image analysis software program (Kevex, San Carlos, CA). Fields of starch grains from different samples were viewed with a 40× objective and then digitized and processed for image analysis. Data collected were expressed in Waddell diameter (diameter calculated from a circle with an equivalent area to a granule).

Statistical Analysis

A factorial design was used to determine significance of genotypic and planting date effects on DSC parameters, %AM, and HKW. Analysis of variance (ANOVA) and correlation analyses for the data were computed (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Thermal Properties

Significant effects on DSC values as a result of planting date and genotype are shown in Table II. The date of planting had a highly significant effect on values for T_p ($P \leq 0.01$) and a significant effect on ΔH ($P \leq$ O.05) (Fig. 2), which increased with later planting dates. Although not significant, a trend for increased R_n also was observed for each genotype (Fig. 2). The extent to which planting date influenced certain DSC parameters, such as T_o, may have been dependent on genotype. This observation was further demonstrated by the significant interaction between genotype and planting date on T_p (Table II). Previously, White et al (1991) also observed environmental effects on DSC values in which starch of several maize populations had more narrow endotherms when grown in a tropical environment than they had in a temperate environment. These data further support the idea that DSC parameters of starches can be influenced by environmental factors. For example, differences in planting date may alter granule characteristics such as %AM and granule diameter, which may result in the observed changes in DSC values. The ranges for DSC parameters in this study were generally small

TABLE I
Planting and Average Flowering Dates for Two Inbred Lines and an F1 Hybrid

	Flowering Days[a] According to Planting Dates			
Genotype	May 15	May 23	May 30	June 6
Oh43	15	20	27	34
A632	21	25	34	39
Oh43×A632	13	22	28	34

[a] Recorded as the average number of days until self-pollination after June 30.

TABLE II
Mean Squares of Differential Scanning Calorimetry (DSC) Parameters, Amylose Percent (%AM), and 100-Kernel Weight (HKW) for Three Maize Genotypes

		DSC Parameters[a]					
Source	DF	T_o	T_p	R_n	ΔH	%AM	HKW
Replicate	1	0.59	0.09	4.61	0.03	0.59	0.92
Planting date (PD)	3	0.16	1.27**[b]	5.25	0.04**	1.56	23.6*
Genotype (G)	2	4.40**	3.70**	0.93	0.005**	3.61*	33.3*
G × PD	6	1.04	0.71**	0.40	0.20	0.07	16.7
Error	11	0.59	0.05	1.80	0.005	0.59	6.6

[a] Onset of gelatinization (T_o); peak of gelatinization (T_p); range of gelatinization (R_n); enthalpy of gelatinization (ΔH).
[b] Significant at $P \leq 0.05$ (*) and $P \leq 0.01$ (**).

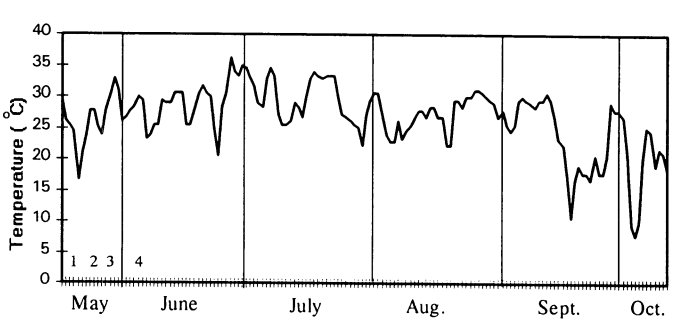

Fig. 1. Daily high temperatures at Ames, IA, for the 1991 growing season in which maize genotypes were planted on May 15, May 23, May 30, and June 6 (1–4, respectively).

compared to the larger ranges among starches from endosperm mutants such as *ae*, *du*$_1$, *su*$_2$, and *wx*, as demonstrated in previous studies (Brockett et al 1988, Sanders et al 1990, Wang et al 1992).

Many significant genotypic effects were seen among the DSC parameters, including T_o, T_p, and ΔH (Table II). Oh43 consistently showed greater values for these parameters than did A632 across each planting date (Fig. 2). Generally, the hybrid Oh43×A632 remained intermediate for these measures relative to the parent inbreds for most planting dates. The heterogeneous nature of segregating kernels collected from the hybrid Oh43×A632 might result in increased variability, thus masking trends due to planting date for T_o and T_p. Similar data suggesting genetic variation among nonmutant maize genotypes for DSC parameters have been detected. For example, Krueger et al (1987b) found differences among DSC endotherms of two inbred lines, and suggested that DSC endotherms could be used as a method for identifying inbreds. Other studies have shown wide variations for several DSC parameters within and among several genetically variable maize populations (Li et al 1991, White et al 1991). Although evidence suggests an abundance of genetic variability in thermal properties among exotic sources of maize, clearly there is a need to further investigate variability within commercially available U.S. germ plasm.

Amylose Content

No significant effect of planting date was seen on %AM for the three genotypes (Table II). These results are in agreement with Williams et al (1958), who found no relationship between %AM and date of planting among U.S. rice varieties at four dates of planting. However, Helm et al (1968) found that later planting dates were associated with a higher %AM among high-amylose maize genotypes. In the present work, fluctuations in the %AM were parallel among the genotypes. Possibly, environmental factors may have influenced %AM of each genotype in a similar manner during the growing season (Fig. 3). This observation may have been the effect of transient temperature changes during the growing season, especially during grain filling.

A significant effect of genotype on %AM was found (Table II). Genotypes ranked consistently in %AM across each planting date (Fig. 3). The influence of genotype among nonmutant sources on %AM also has been reported. Previous studies have shown differences in %AM among collections of normal (nonmutant) accessions of maize (Deatherage et al 1955). Environmental effects, however, may have resulted in the differences because the collections were not necessarily grown in similar environments.

100-Kernel Weight

HKW was significantly affected by both date of planting and genotype (Table II). Later planting dates were associated with decreased HKW for genotypes Oh43 and Oh43×A632, whereas A632 was less affected (Fig. 3). The low HKW for A632 may be due to a later flowering date than that of the other genotypes (Table I). The HKW of Oh43×A632 generally exceeded those of the inbred parents, which is likely a result of the increased vigor of the hybrid genotype.

Starch Granule Size

Average starch granule diameters are shown in Table III for starches of each genotype collected from the first and fourth planting dates. Average granule diameters among the genotypes from these two planting dates ranged from 5.4 to 6.3 μm. No significant differences in granule diameter were observed among starches from the different planting dates. In contrast to the results seen in this study, previous studies with rice have indicated that environment may affect starch granule size (Kongseree and Juliano 1972).

There is some indication that differences among endotherms of nonmutant maize starches may be explained by differences in average granule size. For example, Knutson et al (1982) found a slight broadening and flattening of endotherms for smaller granules when fractionated by sedimentation as compared to those of larger granules. In our study, there was no evidence to support a relationship between starch granule size and shape of the endotherm.

Correlation Analysis

Correlation analyses were conducted among DSC values, %AM, HKW, and flowering date (Table IV). Correlations among several DSC parameters such as T_o and T_p were highly significant ($P \leq 0.01$), indicating that a certain degree of redundancy may exist among these measures. A highly significant reduction in HKW occurred for the later flowering date, as indicated by the negative correlation. A highly significant negative correlation also was seen between flowering date and R_n (Table II). Differences in days to flowering among genotypes within planting dates may have confounded detection of this effect in the ANOVA. Although

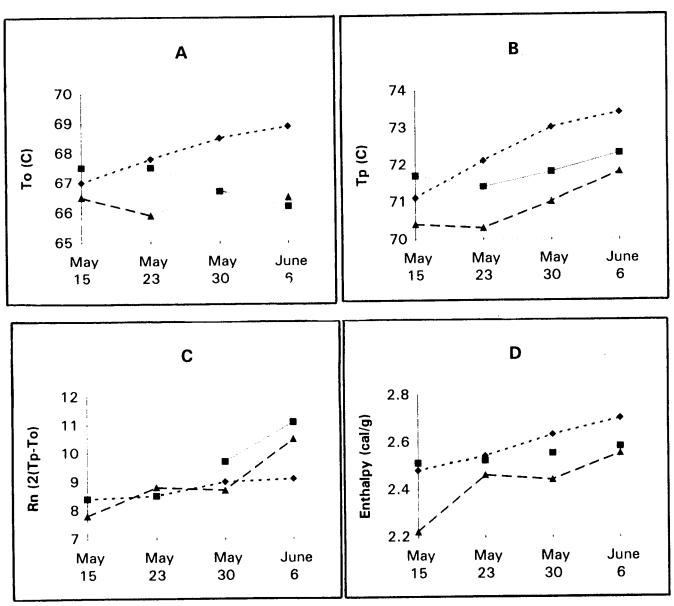

Fig. 2. Genotypes Oh43 (◆), A632 (▲), and Oh43×A632 (■) planted on May 15, May 23, May 30, and June 6. **A,** Mean onset temperature (T_o). **B,** Gelatinization peak temperature (T_p). **C,** Range of gelatinizatrion (R_n). **D,** Total enthalpy of gelatinization (ΔH).

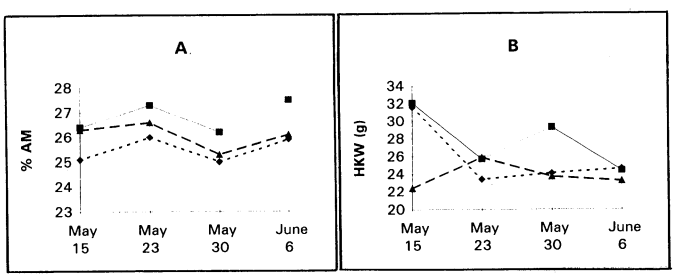

Fig. 3. Genotypes Oh43 (◆), A632 (▲), and Oh43×A632 (■) planted on May 15, May 23, May 30, and June 6. **A,** Mean amylose content (%AM). **B,** 100-kernel weight (HKW).

TABLE III
Mean Starch Granule Diameter and Standard Deviation (SD) for Starch of Oh43, A632, and Oh43×A632

	Planting Date 1 (May 15)			Planting Date 4 (June 6)		
Genotype	Number of granules	Mean (μm)	SD	Number of Granules	Mean (μm)	SD
Oh43	243	5.8	1.6	203	5.4	1.6
Oh43×A632	215	5.4	1.7	218	5.6	1.8
A632	251	5.9	2.0	208	6.3	1.9

TABLE IV
Correlation Analysis of Differential Scanning Calorimetry (DSC) Parameters,[a] 100-Kernel Weight (HKW), Flowering Date (FD), and Percent Amylose (%AM) from Genotypes Planted at Four Dates

	T_p	R_n	ΔH	HKW	FD	%AM
T_o	0.74***[b]	−0.53**	0.46*	−0.02	−0.17	−0.38
T_p	...	0.17	0.70**	−0.02	0.22	−0.17
R_n	...	...	0.20	0.01	0.52**	0.34
ΔH	...	...	...	0.02	0.27	−0.10
HKW	...	...	...	...	−0.53**	−0.15
FD	...	...	...	...	...	0.08

[a]Onset of gelatinization (T_o); peak gelatinization (T_p); range of gelatinization (R_n); enthalpy of gelatinization (ΔH).
[b]Significant at $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.0001$ (***).

the %AM did not greatly influence any DSC parameters, a negative correlation with T_o did approach significance ($P = 0.07$).

CONCLUSIONS

Planting date and genotype affected DSC parameters in this study. Differences in DSC parameters were not large, but the effects on several parameters were highly significant, thus demonstrating the extreme sensitivity of this method for determining differences among starch thermal properties. Increases in T_p, ΔH, and R_n for starches of all genotypes generally were observed with later planting dates. The DSC values were strongly influenced by genotype; starch from Oh43 had greater T_o, T_p, and ΔH values than did starch from A632. The R_n also was significantly correlated with the number of days to flowering. There was no indication that planting date or genotypic differences in starch granule size accounted for differences in thermal properties among the starches. Differences among planting dates are likely because of environmental fluctuations, such as variations in daily high temperatures (Fig. 1), as well as day length during vegetative growth and the grain-filling period.

The present work suggests that the DSC is a good tool to use for screening starches with unusual properties. Further studies are required to determine the extent to which the differences in DSC values reflect differences in functional properties of starches. Future use of DSC in programs designed to screen and select germ plasm may require a more thorough examination of the potential influence of environmental effects and the interaction of these effects with genotypic differences.

LITERATURE CITED

BEACHELL, H. M., and STANSEL, J. W. 1963. Selecting rice for specific cooking characteristics in a breeding program. Int. Rice Commun. Newsl. (Spec. Issue):225-40.

BROCKETT, E., THOMPSON, D., DAVIS, T., and BOYER, C. 1988. Gelatinization characterization of starch from *du, wx, ae* and *ae wx* endosperm of sweet corn inbred Ia 5125. Starch/Staerke 40:175.

DEATHERAGE, W. L., MacMASTERS, M. M., and RIST, C. E. 1955. A partial survey of amylose content in starch from domestic and foreign varieties of corn, wheat and sorghum and from some other starch bearing plants. Trans. Am. Assoc. Cereal Chem. 13:31.

DUNN, G. M., KRAMER, H. H., and WHISTLER, R. L. 1953. Gene dosage effects on corn endosperm carbohydrates. Agron. J. 45:101.

HELM, J. L., FERGESAN, V. L., and ZUBER, M. S. 1968. Effect of planting date on high amylose corn (*Zea mays* L.). Agron. J. 60:530

JULIANO, B. O., NAZARENO, M. B., and ROMOS, N. B. 1969. Properties of waxy and isogenic non-waxy rices differing in starch gelatinization temperature. J. Agric. Food Chem. 17:1364.

KNUTSON, C. A., KHOO, U., CLUSKEY, J. E., and INGLETT, G. E. 1982. Variation in enzyme digestibility and gelatinization behavior of corn starch granule fractions. Cereal Chem. 59:512.

KNUTSON, C. A. 1986. A simplified colorimetric procedure for determination of amylose in maize starches. Cereal Chem. 63:89.

KONGSEREE, N., and JULIANO, B. O. 1972. Physiochemical properties of rice grain and starch from starch differing in amylose content and gelatinization temperature. J. Agric. Food Chem. 20:714.

KRUEGER, B. R., WALKER, C. E., KNUTSON, C. A., and INGLETT, G. E. 1987a. Differential scanning calorimetry of raw and annealed starch isolated from normal and mutant maize genotypes. Cereal Chem. 64:187.

KRUEGER, B. R., KNUTSON, C. A., INGLETT, G. E., and WALKER, J. 1987b. A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. Food Sci. 52:715.

LEMPIANNEN, T., and HENRIKSNAS, H. 1979. Characterization of the starch in the tubers of dropwort (*Filipendula vulgaris* moench). Starch/Staerke 31:45.

LI, J., BERKE, T. G., and GLOVER, D. V. 1991. Variation for thermal properties of starch in 35 tropical maize populations. (Abstr.) American Society of Agronomy: Madison, WI.

SANDERS, E. B., THOMPSON, D. B., and BOYER, C. D. 1990. Thermal behavior during gelatinization and amylopectin fine structure for selected maize genotypes as expressed in four inbred lines. Cereal Chem. 67:594.

SCHOCH, T. J. 1942. Fractionation of starch by selective precipitation with butanol. J. Am. Chem. Soc. 64:2957.

WANG, Y., WHITE, P., and POLLAK, L. 1992. Thermal and gelling properties of maize mutants from the OH43 inbred line. Cereal Chem. 69:328.

WHITE, P. J., ABBAS, I. R., POLLAK, L. M., and JOHNSON, L. A. 1990. Intra- and interpopulation variability of thermal properties of maize starch. Cereal Chem. 67:70.

WHITE, P. J., POLLAK, L. M., and BURKHART, S. 1991. Thermal properties of starches from corn grown in temperate and tropical environments. (Abstr.) Cereal Foods World 36:524.

WILLIAMS, V. R., WU, W. T., YSAI, H. Y., and BATES, H. G. 1958. Varietal differences in amylose content of rice starch. J. Agric. Food Chem. 6:47.

[Received December 27, 1993. Accepted July 18, 1994.]