Temperature and Cultivar Effects on Soybean Seed Oil and Protein Concentrations

Ernest L. Piper* and Kenneth J. Boote

Department of Agronomy, University of Florida, Gainesville, Florida 32611

ABSTRACT: The soybean [*Glycine max* (L.) Merr.] industry is interested in cultivar and climate effects on seed composition. These factors may underlie the known geographic variation in seed protein and oil concentrations. Regression analyses were used to test hypotheses of the effect of temperature and cultivar on oil and protein concentrations of soybean seed using a large data set from the U.S.A. Soybean Uniform Tests. The data set included 20 cultivars representing 10 maturity groups across 60 locations (latitude 29.4 to 47.5° N) for a total of 1863 cultivar by location by year observations. Temperature was determined for each observation as the average daily mean temperature from predicted first pod (first pod at least 5 mm long), using the SOYGRO phenology model, to observed maturity. The mean temperature ranged from 14.6 to 28.7°C among the observations. Linear, quadratic, and linear plateau regression models of oil and protein concentrations vs. temperature were evaluated. The quadratic model gave the best-adjusted R^2 values for oil and protein with temperature, of 0.239 and 0.003, respectively. The analyses showed that the oil concentration increased with increasing temperature and approached a maximum at a mean temperature of 28°C. Unaccounted variation in the protein concentration may be from other factors such as photoperiod, water stress, or high temperatures during seed fill. Protein plus oil had a linear relationship with temperature (adjusted partial R^2 = 0.183). These data document the contribution of climate and cultivar to geographic variability of oil and protein concentrations in the United States.

Paper no. J9114 in *JAOCS 76,* 1233–1241 (October 1999).

KEY WORDS: Genetic variation, genotype by environment interaction, geographic variability, *Glycine max,* modeling, seed composition, seed quality, soybean.

For commercial considerations, knowledge of the underlying causes of geographic variation in seed composition is of great interest to the soybean processing industry. Oil and protein are the major economic products from soybean seed. However, the concentration of oil and protein may range from 120 to 230 g kg^{-1} and 255 to 589 g kg^{-1}, respectively (1). Although this variation may be assumed to be genetic or environmentally induced, the nature of the plant response is not well understood.

*To whom correspondence should be addressed at Department of Agronomy, Iowa State University, 1563 Agronomy Hall, Ames, IA 50011. E-mail: epiper@iastate.edu

The consistent well-documented negative correlation between oil and protein indicates that the oil concentration tends to decrease as the protein concentration increases (2–5). This response has been attributed to both environment and genotypic variation (2–4). Hurburgh *et al*. (6) reported that northern and western locations in the Midwest had a greater protein loss for each concentration point gain in oil than southern locations, yet the negative correlation between protein vs. oil concentration persists at high temperature (7).

Variability in slopes of oil or protein concentrations vs. temperature appears to depend on the temperature range in the study. Keirstead (8) found a significant positive correlation between mean temperature and oil concentration, with oil concentration increasing 7.4 and 8.5 g kg^{-1} $°C^{-1}$ during two growing seasons. Serretti (9) reported that oil concentration increased 6.7 g kg^{-1} $°C^{-1}$. Kane *et al.* (10) found that oil concentration increased 5.2 to 6.6 g kg^{-1} $°C^{-1}$ among six cultivars. Protein concentration had no significant relationship with temperature in these studies (9,10).

Sato and Ikeda (11) and Wolf *et al*. (12) evaluated final oil, protein, soluble sugar, and starch concentrations under a wide range of temperatures in controlled environmental chambers. In their studies, the oil concentration increased with temperature and reached a plateau at mean temperatures above 22°C. The starch and soluble sugar concentrations decreased with increasing temperature and decreased dramatically at mean temperatures greater than 20°C (11). Protein concentration appeared to be relatively constant at lower temperatures but increased at the highest temperature.

Growth chamber and greenhouse experiments have been conducted to establish the response of oil and protein concentration to temperature. Gibson and Mullen (7) and Dornbos and Mullen (13) found that oil content increased with increasing temperature with an optimum at 25 to 28°C, above which the oil concentration declined. The protein concentration was either constant or only slightly increased with decreasing mean temperature below 28°C (7,11,12). At temperatures greater than 28°C, protein concentration increased linearly with temperature (7,13). In addition to temperature, shortening day length may enhance the protein concentration by increasing the rate of nitrogen translocation to the seed and seed growth rate (14). Protein plus oil appears to increase linearly with increasing mean temperature (7,11,12).

Geographic patterns of oil and protein levels of soybean seed reported in the United States show that protein is higher and oil lower in the Southeast and Delta states compared to the Midwest (5,8). Breene *et al*. (15) also found that the protein concentration tended to be less at northern vs. southern locations (34 to 44° N latitude). Northern and northwestern states have been reported to have about 5 g kg^{-1} higher oil and 15 to 20 g kg^{-1} lower protein concentrations than southern states (6). The contribution of cultivar to the observed geographic patterns of seed composition has not been well defined. However, during the past 40 yr, new cultivars in northern states and Canada have increased oil concentration and decreased protein concentration as yield has been improved in these cooler environments (16,17).

Studies of seed composition have been conducted over a limited range of environments. No study in the literature was found that evaluated the response of oil and protein concentrations to temperature across all latitudes in the United States. Evaluation of responses in a large field-data set covering a wide range of temperatures would be desirable for comparison with those observed in controlled environment studies. The objective of this paper is to test hypotheses of the effect of temperature and cultivar on oil and protein composition of soybean seed using a large data set from the Uniform Soybean Tests.

MATERIAL AND METHODS

Cultivar trial data were obtained from "The Uniform Soybean Tests: Northern States and Southern States" publications from the years 1970 to 1990 for over 60 locations ranging in latitude from 47.48 to 29.36° N (Tables 1,2). The Uniform Soybean Testing Program tests elite breeding lines from federal and state research programs for potential as cultivar releases. The trial data included check cultivars that are often grown for many years in the trials. Twenty check cultivars were selected from the Uniform Test data to represent 10 maturity groups (00–VIII). The selected cultivars were McCall (00), Clay (0), Evans (0), Hardin (I), Hodgson (I), Corsoy (II), Century (II), Pella (III), Cumberland (III), Williams (III), Union (IV), Douglas (IV), Essex (V), Forrest (V), Centennial (VI), Braxton (VII), Bragg (VII), Ransom (VII), Hutton (VIII), and Cobb (VIII). Maturity group is given in parentheses. Information reported in The Uniform Tests publications includes oil and protein concentrations (dry weight basis), maturity date, and seed yield.

Daily maximal and minimal temperatures (°C) were obtained from Earth Info, Inc. (NCDC Summary of the Day, Earth Info, Inc., Boulder, CO) CD-ROM disks that contained the United States National Weather Service data (18). The average daily mean temperature and the average daily photoperiod for each observation were determined from first pod, as predicted by the SOYGRO phenology model (19), to the observed maturity date.

Oil concentration vs. the average mean air temperature during seed fill was initially plotted for each cultivar to identify outliers that may bias the regression analysis. As an example, oil concentrations were especially low in 1982 throughout the Midwestern states despite warm temperatures. Thirteen location–year combinations were deleted when the

TABLE 1
Descriptive Statistics of the Uniform Test Data for Percentage Oil, Percentage Protein, and Percentage Protein Plus Oil

		Oil (%)				Protein (%)				Protein plus oil (%)			
Cultivar (maturity group)	*N*	Minimum	Maximum	Mean	Standard deviation	Minimum	Maximum	Mean	Standard deviation	Minimum	Maximum	Mean	Standard deviation
McCall (00)	72	16.9	22.8	20.0	1.45	35.9	44.8	39.7	1.64	56.8	63.2	60.0	1.34
Clay (0)	77	15.4	24.7	20.6	1.90	36.9	44.9	40.9	1.61	56.2	65.1	61.5	1.85
Evans (0)	78	18.1	24.7	20.9	1.72	36.2	43.6	39.7	1.76	56.7	64.1	60.6	1.65
Hodgson (I)	106	16.4	25.3	21.2	2.03	35.5	44.0	39.6	1.82	56.3	66.5	60.8	1.95
Hardin (I)	59	18.0	24.5	21.7	1.63	36.3	43.0	39.7	1.82	57.6	65.1	61.4	1.74
Corsoy (II)	126	16.1	23.9	21.1	1.73	36.6	44.4	40.3	1.71	55.9	65.9	61.4	1.85
Century (II)	81	16.8	22.9	20.1	1.35	38.3	45.4	42.2	1.68	57.3	67.1	62.2	1.92
Williams (III)	148	18.0	24.2	21.5	1.42	35.7	44.4	40.8	1.43	56.5	65.9	62.3	1.70
Pella (III)	83	17.9	24.6	21.4	1.47	35.0	43.4	39.3	1.59	55.7	63.7	60.6	1.77
Cumberland (III)	52	18.3	24.5	21.7	1.63	36.2	44.5	40.5	1.85	58.6	64.9	62.1	1.64
Union (IV)	57	17.4	22.2	20.3	1.10	38.5	46.2	41.9	1.57	58.5	65.9	62.2	1.72
Douglas (IV)	97	16.5	23.1	20.8	1.29	38.2	44.9	41.6	1.61	57.6	67.2	62.3	1.85
Essex (V)	158	17.9	25.0	20.7	1.35	37.5	46.8	41.9	1.81	57.3	65.9	62.6	1.59
Forrest (V)	158	18.1	25.2	21.0	1.51	34.1	45.9	39.6	2.01	53.2	65.4	60.6	2.16
Centennial (VI)	97	16.9	22.4	19.3	1.14	35.6	48.1	42.9	1.97	55.8	67.2	62.2	2.10
Ransom (VII)	78	20.6	25.9	23.3	1.21	34.8	45.0	40.4	1.73	58.6	66.9	63.7	1.61
Braxton (VII)	98	16.8	22.2	19.9	1.13	39.2	46.4	42.0	1.46	59.2	65.6	61.9	1.40
Bragg (VII)	87	18.0	23.8	20.9	1.20	36.9	45.0	41.8	1.53	57.6	67.0	62.7	1.64
Cobb (VIII)	58	18.2	24.0	21.1	1.21	36.9	44.1	40.1	1.83	55.1	65.7	61.2	2.08
Hutton (VIII)	93	17.6	23.5	20.0	1.14	39.6	46.7	43.3	1.50	58.2	67.3	63.4	1.69
Overall	1863	15.4	25.9	20.9	1.65	34.1	48.1	40.9	2.07	53.2	67.3	61.8	2.02

TABLE 2
Descriptive Statistics for Mean Air Temperature, and Night Length During Seed Fill for Each Cultivar Selected from the Uniform Test Data[a]

Cultivar (maturity group)	*N*	Mean temperature (°C)				Night length (h)			
		Minimum	Maximum	Mean	Standard deviation	Minimum	Maximum	Mean	Standard deviation
McCall (00)	72	16.8	24.7	20.8	1.80	8.81	10.34	9.55	0.33
Clay (0)	77	14.6	23.7	20.3	1.89	8.90	10.83	9.77	0.37
Evans (0)	78	16.0	25.2	20.5	2.00	9.36	10.69	9.98	0.31
Hodgson (I)	106	15.2	25.2	20.6	2.21	9.61	11.17	10.15	0.32
Hardin (I)	59	16.8	27.2	22.4	2.55	9.38	10.86	10.06	0.32
Corsoy (II)	126	16.4	27.1	22.0	1.87	9.78	11.14	10.28	0.27
Century (II)	81	18.8	28.7	23.1	2.04	9.73	10.69	10.20	0.20
Williams (III)	148	19.1	28.7	23.3	2.02	10.00	11.38	10.52	0.23
Pella (III)	83	18.9	28.5	23.2	1.92	9.86	10.80	10.32	0.19
Cumberland (III)	52	20.5	28.3	23.6	1.76	9.89	10.82	10.37	0.17
Union (IV)	57	20.4	27.7	23.6	1.92	10.02	11.03	10.56	0.19
Douglas (IV)	97	20.2	28.3	24.2	1.87	10.09	11.44	10.80	0.27
Essex (V)	158	19.3	28.6	24.0	1.98	10.70	11.82	11.23	0.25
Forrest (V)	158	18.7	28.0	23.6	2.10	10.73	11.95	11.32	0.26
Centennial (VI)	97	18.3	27.5	22.6	2.22	11.25	12.19	11.70	0.19
Ransom (VII)	78	20.4	27.9	24.5	1.95	11.24	12.19	11.66	0.20
Braxton (VII)	98	19.9	27.2	24.1	1.62	11.25	12.04	11.62	0.17
Bragg (VII)	87	20.1	28.1	24.7	1.84	11.07	12.15	11.51	0.20
Cobb (VIII)	58	20.1	26.7	23.9	1.70	11.35	12.29	11.82	0.20
Hutton (VIII)	93	20.3	27.2	24.4	1.61	11.20	12.25	11.73	0.19
Overall	1863	14.6	28.7	23.0	2.37	8.81	12.29	10.81	0.73

[a]Each temperature and night length observation is the average of daily values from predicted first pod, using the SOYGRO phenology model, to observed maturity.

oil concentration was 4% greater or less than the other data at mean seed fill temperatures above 24°C. These observations were Elora, Ontario-1982; Crookston, MN-1976; Rosemont, MN-1982; Portageville, MO-1980, 1982; Queenstown, MD-1980; Lafayette, IN-1982; Stoneville, MS-1982; Clayton, NC-1977; Blacksville, SC-1972, 1978; Hartsville, SC-1981; Tifton, GA-1982; Jay, FL-1982. Two observation sites, Keiser, AR-1984 and Knoxville, TN-1982, were removed for the cultivar Douglas because the oil was 5% greater than the other observations at the given temperature. The final data set included 1863 cultivar–location–year observations.

The Uniform Test data contained observations over a wide range of temperatures and photoperiod. However, each cultivar in the data set was exposed to only a portion of the temperature and photoperiod range because of the subset of locations where it was grown. Thus, linear regression analyses were performed by cultivar for oil concentration vs. average daily mean temperature during seed fill (temperature), protein concentration vs. temperature, and protein plus oil vs. temperature. These seed constituents also were regressed against cultivars and temperature. The analysis by cultivar provided insight into analyses over all cultivars by showing trends of oil and protein concentrations across latitude. The regression analyses were performed as discussed by Steel and Torrie (20) and Piper *et al.* (21). Statistical analyses were performed according to procedures found in Statistical Analysis System (SAS) version 6.03 (22).

To determine the function of oil, protein, and protein plus oil over the complete temperature range of the Uniform Test data, analyses were conducted using the entire data set, where cultivars were treated as blocks. The "best" model was determined by the largest adjusted R^2, which was calculated as follows:

$$R^2 = \frac{\text{sums of squares regression}}{\text{sums of squares corrected total}} \quad [1]$$

$$\text{adjusted } R^2 = 1 - \left(\frac{n-1}{n-p}\right)\left(1-R^2\right) \quad [2]$$

where p is the number of terms in the model and n is the number of observations (20). Cultivars were treated as blocks with 19 degrees of freedom. The analysis allows the evaluation of cultivar oil and protein concentration relative to the mean of the 20 cultivars, after accounting for the "co-variate effect" of temperature on composition.

Polynomial-linear, polynomial-quadratic, and linear-plateau functions were fitted to the Uniform Test data for oil concentration vs. temperature (7,13). The mathematical representation for the linear regression of oil concentration vs. temperature is

$$y = \alpha_0 + \sum_{i=1}^{19}\sum_{j=1}^{20}\alpha_i C_j + \beta_0 X_{jk} + \varepsilon_{jk} \quad [3]$$

where α_0 is the intercept, each α_i has a value of 0, 1, or –1 for the corresponding C_j cultivar contribution to the intercept, β_0 is the linear slope component, X_{jk} is the temperature for the jth cultivar's kth observation , and ε_{jk} is the random error associated with the kth observation of the jth cultivar.

The mathematical representation of the quadratic model is

$$y = \alpha_0 + \sum_{i=1}^{19} \sum_{j=1}^{20} \alpha_i C_j + \beta_0 X_{jk} + \gamma_0 X_{jk}^2 + \varepsilon_{jk} \quad [4]$$

where α_0 is the intercept, each α_i has a value of 0, 1, or −1 for the corresponding C_j cultivar contribution to the intercept, β_0 is the linear slope component, γ_0 is the quadratic slope component, X_{jk} is the temperature for the *j*th cultivar's *k*th observation, and ε_{jk} is the random error associated with the *j*th cultivar's *k*th observation.

The model for the linear plateau function is

$$y = \alpha_0 + \sum_{i=1}^{19} \sum_{j=1}^{20} \alpha_i C_j + \beta_0 X_{jk} + \varepsilon_{jk} \quad \text{for } X_i \le t \quad [5]$$

$$y = \alpha_0 + \sum_{i=1}^{19} \sum_{j=1}^{20} \alpha_i C_j + \beta_0 t + \varepsilon_{jk} \quad \text{for } X_i > t \quad [6]$$

where α_0 is the effect of the intercept, each α_i has a value of 0, 1, or −1 for the corresponding C_j cultivar contribution to the intercept, β_0 is the linear component, X_{jk} is the temperature for the *j*th cultivar and *k*th observation, t is the joint point between the linear slope and the plateau, and ε_{jk} is the random error associated with the *j*th cultivar's *k*th observation.

Linear and quadratic models of protein concentration vs. temperature were evaluated, and a linear model was evaluated for protein plus oil. These models are similar to the linear and quadratic models for percentage oil presented above. In addition, a two-phase linear model was evaluated to test the hypothesis that protein plus oil may not be linear at high temperatures in field situations. This model differs from the linear plateau model in that the second phase can have a positive or negative slope. The model for the two-phase linear model is

$$y_i = \alpha_0 + \sum_{i=1}^{19} \sum_{j=1}^{20} \alpha_i C_j + \beta_0 t + X_{jk} + \varepsilon_{jk} \quad \text{for } X_i \le t \quad [7]$$

$$y_i = \alpha_0 + \sum_{i=1}^{19} \sum_{j=1}^{20} \alpha_i C_j + \beta_0 t + \beta_1 \left(X_{jk} - t \right) + \varepsilon_{jk} \quad \text{for } X_{jk} > t \quad [8]$$

where α_0 is the intercept, each α_i has a value of 0, 1, or −1 for the corresponding C_j cultivar contribution to the intercept, β_0 is the slope of the first phase, β_1 is the slope of the second phase, X_{jk} is the temperature for the *j*th cultivar and *k*th observation, and ε_{jk} is the random error associated with the *j*th cultivar's *k*th observation.

RESULTS AND DISCUSSION

Preliminary analyses indicated that mean temperature, rather than maximal or minimal temperature, resulted in the highest correlations and best regression relationships of oil and protein concentrations vs. temperature. This agrees with the results of Gibson and Mullen (7) who found that seed oil and protein concentrations were affected by both day and night temperature. Mean temperature was used for all analyses.

Evaluation of the Uniform Test data sets. The minimum, maximum, mean, and standard deviation for the parameters measured from the Uniform Test cultivars are shown in Tables 1 and 2. Although there was considerable range in oil, protein, protein plus oil, and temperature for each cultivar, the standard deviations within cultivars indicated central tendencies with less frequency at the extremes. The variation was as high as 10% for oil and 8% for protein for a single cultivar (Table 1). Although the Uniform Test data include many observations for 20 cultivars, the cooler environments included only the earliest maturity cultivars while the warmest environments included only the latest maturity cultivars (Table 2). Standard deviations of temperature and observed oil and protein percentages are especially small for the two cultivars in maturity group IV.

The range in night length of each cultivar corresponded to its photoperiod sensitivity and latitude range of adaptation. Pearson correlation coefficients indicated highly significant negative correlations between temperature and night length among cultivars, ranging from −0.17 to −0.78 (not shown). Owing to these correlations, the effect of night length on seed composition could not be evaluated separately with the Uniform Test data. When data have a central tendency, regression analysis results can be more difficult to interpret, as the central portion of the range is weighted more heavily than the extremes. With knowledge of the biological system and careful judgment, appropriate terms to include in a model can be determined and useful information can be gained of the nature of the response.

Assumption of no cultivar × environment interaction. No important cultivar × temperature interactions, as indicated by a change of ranking, have been reported in the literature for conventional cultivars (23–26). High protein lines are known to vary in response to temperature and photoperiod (14). We conducted a stability analysis to test the null hypothesis: All cultivars maintain their ranking across environments with respect to seed oil and protein composition. This procedure requires that all treatments (in this case, cultivars) be present at every environment (location–year combination) so the environmental index (average of a variable for each location and year combination) can be calculated for the analysis (27,28). Five suitable data sets were formed from the Uniform Test data: the first data set included four cultivars at seven locations for a total of 136 observations, the second data set included four cultivars at three locations for a total of 40 observations, the third data set included three cultivars at three locations for a total of 51 observations, the fourth data set included six cultivars at three locations for a total of 60 observations, and the fifth data set included four cultivars at four locations for a total of 60 observations. The total number of observations depended on the number of years of data, which varied between locations. In all cases the cultivar × environment index was nonsignificant at the 0.05 percent probability level, supporting the null hypothesis (data not shown). Thus, composition of the selected 20 cultivars from the Uniform Tests appeared to have responded similarly to temperature and photoperiod, at least in the temperature and photoperiod ranges experienced in this data set.

TABLE 3
The Pearson Correlation Coefficients for Percentage Oil vs. Protein

Cultivar (maturity group)	Oil vs. protein	*P*-value[a]	Cultivar	Oil vs. protein	*P*-value
McCall (00)	–0.6289	0.0001	Union (IV)	–0.2116	0.1141
Clay (0)	–0.4528	0.0001	Douglas (IV)	–0.2048	0.0442
Evans (0)	–0.5540	0.0001	Essex (V)	–0.5270	0.0001
Hodgson (I)	–0.4935	0.0001	Forrest (V)	–0.2733	0.0005
Hardin (I)	–0.4974	0.0001	Centennial (VI)	–0.1649	0.1065
Corsoy (II)	–0.4208	0.0001	Ransom (VII)	–0.4499	0.0001
Century (II)	–0.2121	0.0573	Braxton (VII)	–0.4436	0.0001
Williams (III)	–0.2902	0.0003	Bragg (VII)	–0.2924	0.0060
Pella (III)	–0.3387	0.0017	Cobb (VIII)	–0.1042	0.4365
Cumberland (III)	–0.5639	0.0001	Hutton (VIII)	–0.2010	0.0534
Overall	–0.4273	0.0001			

[a]The probability that the population value of the Pearson correlation coefficient is 0 (H_0: Rho = 0), i.e., no relationship between oil and protein concentration.

Analyses by cultivar. (i) The correlation of oil vs. protein concentration. Researchers have reported a consistent negative correlation between oil and protein concentrations (4,7,29). The correlation of oil vs. protein over the entire Uniform Test data was –0.4273 ($P < 0.0001$) (Table 3). Similar to the correlations reported by Cartter and Hopper (23), the analysis by cultivar shows that the correlation coefficients tended to decrease with warmer temperatures at more southern latitudes. However, the correlations of Braxton, Ransom, and Essex were similar to the earlier maturity groups.

(ii) Linear regression analyses. Dornbos and Mullen (13) and Gibson and Mullen (7) found that oil concentration vs. temperature had a quadratic relationship with temperature and decreased above the optimal temperature of 28°C. If oil increase has a quadratic relationship with temperature, the slope of oil vs. temperature from a linear regression should decrease as one moves from northern to southern latitudes. Indeed, the Uniform Test data indicate that the slope of oil vs. temperature tends to decrease with decreasing latitude (Table 4).

Two cultivars, Williams and Douglas, had much smaller slopes of oil concentration vs. temperature than the others. Data from these cultivars were shown in plots to have a strong central tendency in the temperature range and large scatter in the data (not shown). The small slopes of the fitted regression line for Williams and Douglas are probably the consequence of the central tendency of the observed data and not cultivar

TABLE 4
The Linear Regression of Percentage Oil, Percentage Protein, and Percentage Protein Plus Oil, Each vs. Mean Temperature[a]

Cultivar (maturity group)	Oil (%)		Protein (%)		Protein plus oil (%)	
	Intercept	Slope[b]	Intercept	Slope[b]	Intercept	Slope[b]
McCall (00)	9.14	0.5212**	46.19	–0.3137**	55.33	0.2075*
Clay (0)	4.96	0.7711**	46.14	–0.2601**	51.11	0.5110**
Evans (0)	8.94	0.5838**	46.29	–0.3207**	55.23	0.2631**
Hodgson (I)	6.94	0.6913**	44.98	–0.2633**	51.92	0.4280**
Hardin (I)	10.46	0.5045**	42.15	–0.1106	52.61	0.3939**
Corsoy (II)	8.21	0.5888**	43.96	–0.1663*	52.17	0.4225**
Century (II)	12.15	0.3437**	38.70	0.1501	50.85	0.4938**
Williams (III)	16.96	0.1940**	39.49	0.0581	56.44	0.2520**
Pella (III)	11.38	0.4305**	38.98	0.0128	50.36	0.4433**
Cumberland (III)	8.03	0.5764**	44.62	–0.1753	52.64	0.4011**
Union (IV)	14.89	0.2296**	39.80	0.0877	54.69	0.3172**
Douglas (IV)	16.27	0.1860**	36.83	0.1948*	53.10	0.3808**
Essex (V)	14.90	0.2438**	39.49	0.0985	54.40	0.3424**
Forrest (V)	13.82	0.3029**	29.79	0.4143**	43.61	0.7172**
Centennial (VI)	12.90	0.2837**	36.34	0.2884**	49.24	0.5721**
Ransom (VII)	15.44	0.3203**	35.75	0.1877	51.20	0.5080**
Braxton (VII)	12.72	0.2969**	39.18	0.1165	51.90	0.4134**
Bragg (VII)	14.25	0.2680**	36.02	0.2336**	50.27	0.5017**
Cobb (VIII)	13.05	0.3348**	32.63	0.3142*	45.69	0.6490**
Hutton (VIII)	13.02	0.2867**	37.83	0.2267*	50.86	0.5133**

[a]Each temperature observation is the average of daily values of mean temperature from predicted first pod, using the SOYGRO phenology model, to observed maturity.
[b]*, ** indicates significance at the 0.05 and 0.01 probability levels, respectively.

differences. The differences in slope for Williams and Douglas compared to the other cultivars are not considered real.

Previous correlations in the literature of protein concentration with temperature have indicated that protein either decreases or has no relationship with temperature (9,10,24,29). The linear regression analysis of the slope of protein concentration vs. temperature from the Uniform Test data indicated that the slope was negative at northern latitudes, the slope was not significant at mid-latitudes, and the slope tended to be significant and positive at southern latitudes (Table 4). A negative slope of protein concentration at lower temperature shown for the cultivar Evans (Fig. 1A) is in contrast to the chamber studies of Sato and Ikeda (11) and Wolf *et al.* (12). The literature indicates that the protein concentration should decline at lower temperatures (7). The chamber studies of Gibson and Mullen (7) and Dornbos and Mullen (13) support a linear increase in protein concentration with increasing temperature as was observed for cultivars Forrest and Hutton (Fig. 1B,C). The cause of different responses for protein among cultivar trial data is unknown. All the cited studies were chamber studies where selected temperatures were held constant for day and night, as opposed to natural diurnal cycles.

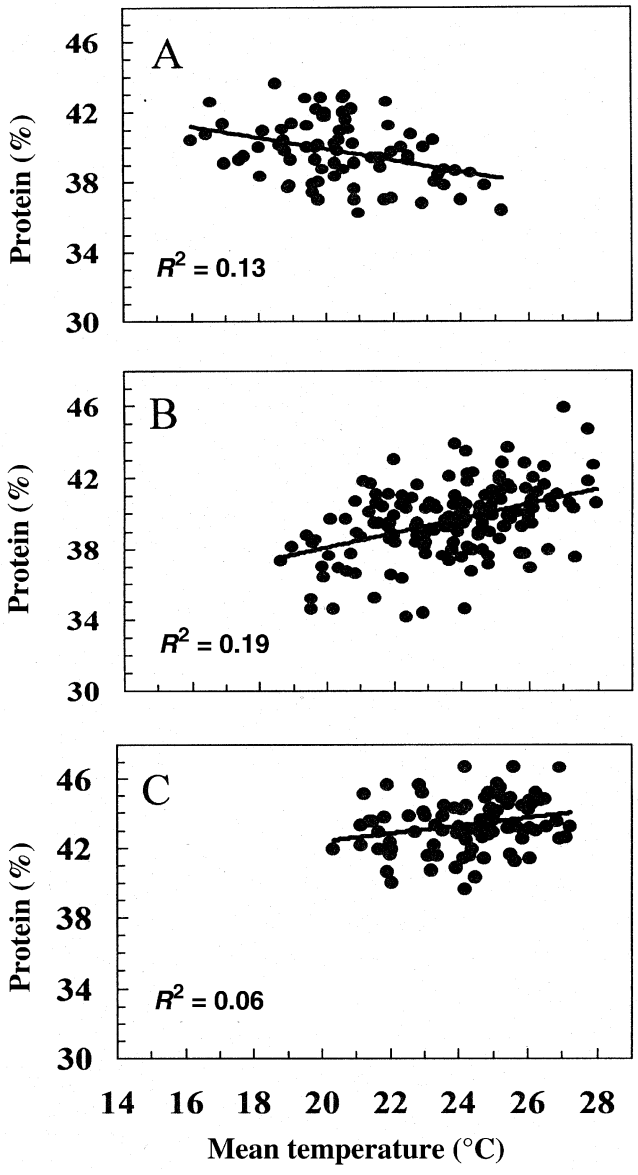

FIG. 1. Protein (%) vs. temperature during seed fill for soybean cultivars Evans (A), Forrest (B), and Hutton (C). Each temperature observation is the average of daily values of mean temperature from predicted first pod, using the SOYGRO phenology model, to observed maturity. See text for further explanation.

All cultivars had positive slopes of protein plus oil concentrations vs. temperature with a range from 0.21 to 0.72% $°C^{-1}$ (Table 4). This concurs with the results of Sato and Ikeda (11) and Wolf *et al.* (12) where protein plus oil had a linear relationship with temperature. Cultivar variation in slope is probably not significant considering the scatter in the data and the narrow range in temperature for some cultivars.

Analysis of the whole data set. The Uniform Test data were analyzed next with cultivars treated as blocks. Treating cultivars as blocks implies the assumption that cultivars respond similarly to temperature with respect to seed composition. The results of the analyses of percentage oil, percentage protein, and protein plus oil each vs. temperature are shown in Table 5.

(i) Oil. The adjusted R^2 values for the linear, quadratic, and linear-plateau regression of oil on temperature indicated that the quadratic model best fit the data. Cultivar and temperature accounted for equal portions of the variation in the oil concentration. The slope of oil vs. temperature was 0.39% $°C^{-1}$ for the linear model and 0.53% $°C^{-1}$ for the linear plateau model. The joint point for the linear plateau was estimated to be at 24.29°C. This temperature is lower than the optimal temperature range of 26–29°C reported by Dornbos and Mullen (13) and Gibson and Mullen (7). The slope for the quadratic model decreased from 1.01% $°C^{-1}$ at 15°C to 0.61% $°C^{-1}$ at 20°C and 0.21% $°C^{-1}$ at 25°C (Fig. 2). The optimal temperature with the quadratic model occurred at 27.7°C, which falls within the range observed in chamber studies (7,13).

TABLE 5
The Adjusted R^2 Values for Various Functions for Percentage Oil, Percentage Protein, and Protein Plus Oil, Each vs. the Mean Temperature During Seed Fill

		Partial adjusted R^2	
Function	Adjusted R^2	Cultivar	Temperature[a]
Oil			
Linear	0.4311**[,b]	0.2213**	0.2098**
Quadratic	0.4602**	0.2213**	0.2389**
Linear plateau	0.4523**	—	—
Protein			
Linear	0.3198**	0.3171**	0.0027**
Quadratic	0.3337**	0.3171**	0.0166**
Protein plus oil			
Linear	0.4033**	0.2200**	0.1833**

[a]The sum of all temperature variables.
[b]** indicates significance at the 0.01 probability level.

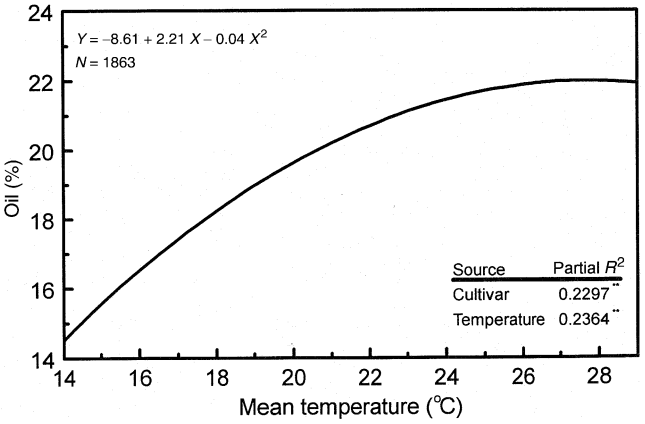

FIG. 2. The response of percentage oil vs. temperature over all soybean cultivars combined. Each temperature observation is the average of daily values of mean temperature from predicted first pod, using the SOYGRO phenology model, to observed maturity. See text for further explanation. **Significant at the 0.01 probability level.

(ii) Protein. A quadratic regression was the best model for protein vs. temperature (Fig. 3). Cultivar and temperature accounted for variation in the protein concentration. Although highly significant, temperature accounted for a small fraction of the variation in the protein concentration. The slope from the linear regression of percentage protein vs. temperature was 0.06% $°C^{-1}$. A quadratic function of protein vs. temperature is consistent with the analysis by cultivar. The quadratic regression supports observations that protein decreases with increasing temperature between 14 and 20°C (23). The leveling off at the mid-temperature range supports the many observations of no relationship between percentage protein and temperature (24,30). The protein concentration increased with temperature above 25°C, agreeing with observations that protein increases at high temperature (7,12,13), and may result from drought stress, which is often associated with higher

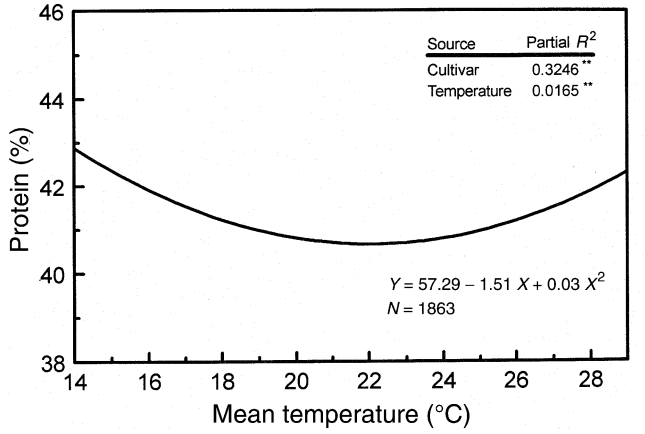

FIG. 3. The response of percentage protein vs. temperature over all soybean cultivars combined. Each temperature observation is the average of daily values of mean temperature from predicted first pod, using the SOYGRO phenology model, to observed maturity. See text for further explanation.

temperatures in field situations (30). The reader should note that the quadratic curve predicts protein concentration to vary about 2%, whereas the observed range within cultivar is about 8%. Temperature obviously did not account for much of the variation in the protein concentration.

The protein response at low temperatures was not consistent with observations in chamber studies (11,12). Protein concentrations in these studies appear to remain constant or slightly increase with decreasing temperature below 28°C (11,12) and increase linearly above 28°C (7,13). The cultivar Evans is shown as an example of the protein concentration increasing with decreasing temperature (Fig. 1A). Why does the protein concentration increase with decreasing temperature between 20 and 14°C? In this temperature range the oil concentration is declining rapidly with temperature, and the protein fraction may increase merely by the summation of components (i.e., the well-known negative relationship between protein and oil) (Fig. 2). Although day length has no direct effect on oil, as a cultivar is planted further north, flowering and seedfill are delayed to occur later in the year when temperatures are cooler. In addition, waning day length may enhance the protein concentration by increasing the rate of nitrogen translocated to the seed (14).

The Uniform Test data are typical of field data in that there is a failure to achieve a uniform orthogonal contrast across all photoperiods and temperatures. Even if all cultivars are grown in the field at the same location, the temperatures and night lengths experienced during seedfill will vary because of cultivar variation in photoperiod sensitivity for date of first pod and maturity. Orthogonal data across all possible temperature and night length combinations may be needed to separate the effects of these two factors to understand why protein is observed to increase with decreasing temperature in the Uniform Test data. Such a study could only be conducted in a growth chamber or greenhouse environment where photoperiod and temperature are controlled.

(iii) Protein plus oil. Response of protein plus oil concentration to temperature was linear with temperature (Fig. 4). Cultivar and temperature accounted for variation. The slope of protein plus oil concentration may decrease at high temperatures (7). When a two-phase function of protein plus oil vs. temperature was tested, the Uniform Test data were linear throughout the range of the data. No curvature in protein plus oil concentration was found at high temperatures. The joint point for the second phase was at a temperature beyond the observed data (not shown). Protein plus oil increased by 0.44% $°C^{-1}$, suggesting that at cooler temperatures, carbohydrate is not used for oil and protein synthesis because enzymes are limiting their rate.

Are regional differences in oil and protein caused by climate or cultivar? These analyses have helped explain whether genetic or environmental factors cause regional differences in oil and protein composition of soybean seed. Temperature-induced shifts in composition were determined in regression analysis where a blocking approach (X matrix) was used to account for individual cultivar effects. Since tempera-

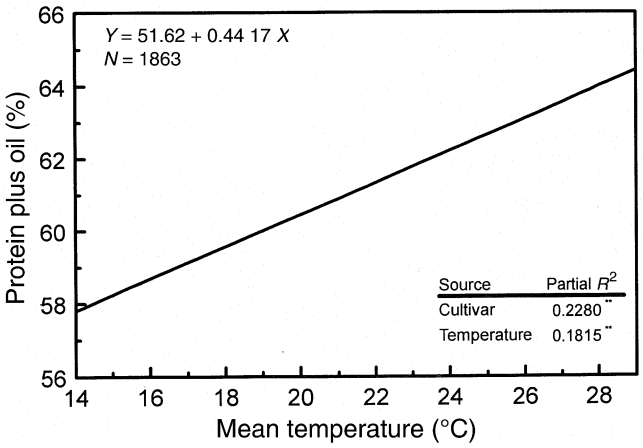

FIG. 4. The response of protein plus oil (%) vs. temperature over all soybean cultivars combined. Each temperature observation is the average of daily values of mean temperature from predicted first pod, using the SOYGRO phenology model, to observed maturity. See text for further explanation.

ture effects were accounted for in a "covariate" manner, this approach led us to the conclusion that the range of true genetic variation was 3.4% for oil, 4.0% for protein, and 2.7% for the protein plus oil, among these adapted cultivars. The northern cultivars in this study have a higher genetic potential for oil concentration than do the southern cultivars, so that the superiority of southern environments is offset and the observed concentrations are similar. This agrees with reports that gain of oil concentration for northern cultivars has increased and is high for these cultivars (16). There is a small but less obvious trend for northern cultivars to have slightly lower genetic potential for protein concentration. Higher protein concentrations in southern soybean cultivars can partially be explained by greater genetic potential for protein and the increase in protein concentration with increasing temperatures. Climatic trends associated with region caused increasing oil concentration and increasing protein plus oil as temperature increased from north to south. It appears that soybean breeders in northern states attempted to overcome the effect of lower temperature on oil by selecting for higher genetic potential for oil but inadvertently also obtained lower genetic potential for protein. Thus, the genetic background of the breeding lines used in cultivar development may influence geographic variability in oil and protein concentration and may be a factor for lower protein concentrations observed in northern locations.

ACKNOWLEDGMENTS

Support for this research was provided by the American Soybean Association, Grant #153223, and is gratefully acknowledged. Florida Experiment Station Journal Series No. R-04066.

REFERENCES

1. Bils, R.F., and R.W. Howell, Biochemical and Cytological Changes in Developing Soybean Cotyledons, *Crop Sci. 3*:304–308 (1963).
2. Hymowitz, T., F.I. Collins, J. Panczer, and W.M. Walker, Relationship Between the Content of Oil, Protein, and Sugar in Soybean Seed, *Agron. J. 64*:613–616 (1972).
3. Watanabe, I., and T. Nagasawa, Appearance and Chemical Composition of Soybean Seeds in Germplasm Collection of Japan II. Correlations Among Protein, Lipid and Carbohydrate Percentage, *Japan Jour. Crop Sci. 59*:661–666 (1990).
4. Weber, C.R., Inheritance and Interrelation of Some Agronomic and Chemical Characters in an Interspecific Cross in Soybeans. *Glycine max* × *G. ussuriensis,* Iowa Agricultural Experiment Station Research Bulletin No. 374, Ames, 1950.
5. Burton, J.W. Quantitative Genetics; Results Relevant to Soybean Breeding, in *Soybeans: Improvement, Production, and Uses*, 2nd edn., Agronomy Monograph No. 16, edited by J.R. Wilcox, Agronomy Society of America–Crop Science Society of America–Soil Science Society of America, Madison, pp. 589–629, 1987.
6. Hurburgh, C.R., Jr., T.J. Brumm, J.M. Guinn, and R.A. Hartwig, Protein and Oil Patterns in U.S. and World Soybean Markets, *J. Am. Oil Chem. Soc. 67*:966–973 (1990).
7. Gibson, L.R., and R.E. Mullen, Soybean Seed Composition Under High Day and Night Growth Temperatures, *Ibid. 73*:733–737 (1996).
8. Keirstead, C.H., Marketing Study of Factors Affecting the Quantity and Value of Products Obtained from Soybeans, U.S. Department of Agriculture, Production and Marketing Administration, U.S. Government Printing Office, Washington, DC, 1952, 35 pp.
9. Serretti, C., Influence of High Protein, Genotype, and Environment on Protein Quality of Soybean, Master's Thesis, Kansas State University, Manhattan, Kansas, 1993.
10. Kane, M.V., C.C. Steele, L.J. Grabau, C.T. MacKown, and D.F. Hildebrand, Early-Maturing Soybean Cropping System: III. Protein and Oil Contents and Oil Composition, *Agron. J. 89*: 464–469 (1997).
11. Sato, K., and T. Ikeda, The Growth Responses of Soybean to Photoperiod and Temperature. IV. The Effect of Temperature During the Ripening Period on the Yield and Characters of Seeds, *Jpn. J. Crop. Sci. 48*:283–290 (1979).
12. Wolf, R.B., J.F. Cavins, R. Kleiman, and L.T. Black, Effect of Temperature on Soybean Seed Constituents: Oil, Protein, Moisture, Fatty Acids, Amino Acids, and Sugars, *J. Am. Oil Chem. Soc. 59*:230–232 (1982).
13. Dornbos, D.L., Jr., and R.E. Mullen, Soybean Seed Protein and Oil Contents and Fatty Acid Composition Adjustments by Drought and Temperature, *Ibid. 69*:228–231 (1992).
14. Cure, J.D., R.P. Patterson, C.D. Raper, Jr., and W.A. Jackson, Assimilate Distribution in Soybeans as Affected by Photoperiod During Seed Development, *Crop Sci. 22*:1245–1250 (1982).
15. Breene, W.M., S. Lin, L. Hardman, and J. Orf, Protein and Oil Content of Soybeans from Different Geographic Locations, *J. Am. Oil Chem. Soc. 65*:1927–1931 (1988).
16. Voldeng, H.D., E.R. Cober, D.J. Home, C. Gillard, and M.J. Morrison, Fifty-eight years of Genetic Improvement of Short-season Soybean Cultivars in Canada, *Crop Sci. 37*:428–431 (1997).
17. Wilcox, J.R., W.T. Schapaugh, Jr., R.L. Bernard, R.L. Cooper, W.R. Fehr, and M.H. Nichaus, Genetic Improvement of Soybean in the Midwest, *Ibid. 19*:803–805 (1979).
18. Piper, E.L., K.J. Boote, and J.W. Jones, Evaluation and Improvement of Crop Models Using Regional Cultivar Trial Data, *App. Eng. Agric. 14*:435–446 (1998).
19. Piper, E.L., K.J. Boote, J.W. Jones, and S.S. Grimm, Comparison of Two Phenology Models for Predicting Flowering and Maturity Date of Soybean, *Crop Sci. 36*:1606–1614 (1996).

20. Steel, R.G.D., and J.H. Torrie, *Principles and Procedures of Statistics,* 2nd edn., McGraw-Hill, New York, 1980, pp. 311–344.
21. Piper, E.L., M.L. Swearingin, and W.E. Nyquist, Postemergence Wheel-Traffic Effects on Plant Population and Yield in Solid-Seeded Soybean, *J. Prod. Agric. 2*:250–256 (1989).
22. SAS, *SAS® Procedures Guide for Personal Computers,* Release 6.03 Edition, SAS Institute Inc., Cary, 1988, 441 pp.
23. Cartter, J.L., and T.H. Hopper, Influence of Variety, Environment, and Fertility Level on the Chemical Composition of Soybean Seed*, USDA Technical Bulletin 787,* U.S. Government Printing Office, Washington, DC, 1942.
24. Howell, R.W., and J.L. Cartter, Physiological Factors Affecting Composition of Soybeans: II. Response of Oil and Other Constituents of Soybeans to Temperature Under Controlled Conditions, *Agron. J. 50*:664–667 (1958).
25. Maestri, D.M., D.O. Labuckas, J.M. Meriles, A.L. Lamarque, J.A. Zygadlo, and C.A. Guzman, Seed Composition of Soybean Cultivars Evaluated in Different Regions, *J. Sci. Food Agric. 77*:494–498 (1998).
26. Serretti, C., W.T. Schapaugh, Jr., and R.C. Leffel, Amino Acid Profile of High Seed Protein Soybean, *Crop Sci. 34*:207–209 (1994).
27. Hildebrand, P.E., Modified Stability Analysis of Farmer-Managed, On-Farm Trials, *Agron. J. 76*:271–274 (1984).
28. Raun, W.R., H.J. Barreto, and R.L. Westerman, Use of Stability Analysis for Long-Term Soil Fertility Experiments, *Ibid. 85*:159–167 (1993).
29. Weiss, M.G., C.R. Weber, L.F. Williams, and A.H. Probst, Correlation of Agronomic Characters and Temperature With Seed Compositional Characters in Soybeans, as Influenced by Variety and Time of Planting, *Ibid. 44*:289–297 (1952).
30. Rose, I.A., Effects of Moisture Stress on the Oil and Protein Components of Soybean Seeds, *Aust. J. Agric. Res. 39*:163–170 (1988).

[Received January 7 1999; accepted May 25, 1999]