# Occurrence and Inheritance of Linolenic and Linoleic Acids in Soybean Seeds<sup>1</sup>

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The range of linolenic acid in the oil from 251 field-grown varieties and introductions of soybeans was 4.89 to 9.28% and that of linoleic, 35.8 to 53.4%.

Crosses of varieties and introductions "high" and "low" in linolenic acid indicated that inheritance of both acids was quantitative rather than qualitative. Transgressive segregation, particularly to low values, was observed occasionally. However 3.35% was the lowest linolenic acid value observed. Environmental effects markedly influenced the quantity of both acids.

 $F_2$  plants from one cross (P.I.80476 x P.I.85671), grown in the greenhouse, showed a dissimilar type of frequency distribution for the two acids but a significant positive correlation (P<1%) between their percentages. A similar relation was not detected for field-grown plants.

THE PERCENTAGE of polyunsaturated fatty acids in oil from soybean seeds has not been used generally as a criterion for selection in breeding programs. However, since there is considerable evidence that linolenic acid may be at least partly responsible for the undesirable flavor reversion occurring in the refined oil (1,2), studies of fatty acid inheritance in soybeans might yield useful information.

Scholfield and Bull (3) reported considerable variability in the percentage of each of the unsaturated fatty acids in soybean oil from 10 varieties grown in different years. Their values for the linolenic acid ranged from 1 to 10% of the total in the oil. Yuskevich (4) reported 0.5 to 12.5% linolenic acid in Russian soybean oils. Studies in recent years have not revealed percentages of linolenic acid comparable with the lowest values found by these earlier workers. Alderks (5), in a study of location composites of 13 varieties, observed ranges of linolenic acid from 6.2 to 8.5% and of linoleic from 49 to 59%. Collins and Howell (6) observed a difference of only about 2.5%between extremes of linolenic acid content for both variety and location composites of seeds from the major area of soybean production in the United States. More recently, the fatty acid composition of 18 varieties from a wider north-to-south range than in the previous study was found to range from 5 to 11% linolenic and from 43 to 56% linoleic acid (7). Of the environmental factors studied by Howell and Collins (8), the maximum temperature during seed development was the most influential on the proportion of linolenic and linoleic acids in the seed oil. The contents of both acids were negatively correlated with temperature.

Data obtained by Simmons and Quackenbush (9), dealing with the sequence of formation of fatty acids in developing soybean seeds, provided evidence that oleic acid may be converted to the more highly unsaturated C<sub>18</sub> acids. This report concerns a study of the proportions of linolenic and linoleic acids in the seed oil of several soybean crosses. Data through the  $F_3$  generation are included.

#### Methods

Soybean introductions obtained from the U.S. Regional Soybean Laboratory at Urbana, Ill., were planted along with commercial varieties at Lafayette, Ind., in 1952, and the harvested seeds were analyzed for their content of linolenic and linoleic acids. Crosses (Table I) were then made in 1953 of varieties Midwest and Harosoy containing high percentages of linolenic acid and the introductions P.I.85671, P.I.68423, and P.I. 80476 containing low percentages of linolenic acid.  $F_1$ ,  $F_2$ , and parent plants were grown together, randomized in two blocks, in a field plot in 1954. All field plants were spaced 4 in. apart in rows 40 in. apart. Each parent,  $\hat{F}_1$ , and  $F_2$  group, was separated by four hills of T135, a chlorophylldeficient type. One T135 seed was planted with all  $F_1$  and  $F_2$  seeds to provide a plant in the hill if the hybrid seed failed to grow.

 $F_3$  lines were field-grown in rows 8 ft. long and 40 in. apart in 1955 and in a single row with a 2-ft. interspace between lines in 1956.

In the winter of 1958–59 plants of cross P.I.80476 x P.I.85671 (Table I) were grown in the greenhouse in 6-in.-in-diameter clay pots with one plant per pot. The day and night temperature in the greenhouse was  $75 \pm 4^{\circ}$ F. Lights (Mazda, 300-watt, in 15-in. reflectors) approximately 15 in. above the plant apex supplied illumination 14 hrs. per day.

Soybean seed samples collected at maturity were stored at 2°C. They were later ground to pass a 20-mesh sieve, placed in Erlenmeyer flasks fitted with glass stoppers, and extracted with ethyl ether with occasional swirling for 24 hrs. in the dark. Fat extraction by this method avoided long periods of continuous heating of the lipid and allowed large numbers of samples to be processed at one time. After decantation from the meal most of the solvent was evaporated on a steam bath, and final traces were removed in a vacuum oven at 50°C. Lipid samples were stored in small centrifuge tubes at  $-20^{\circ}$ C. The samples were brought to room temperature, stirred, and then centrifuged to remove any suspended particles in the oil before analysis.

For the analyses of the introductions and the fieldgrown samples in 1954–56 for linolenic and linoleic acid contents, the lipid samples were isomerized in 11% KOH in glycerol at 180°C. for 45 min. and examined spectrophotometrically (10). The amount of these acids in the oil from seeds matured in the greenhouse was determined spectrophotometrically

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	TA	BLE I					
Composition of Seed Popula	Oil from Sing tions of Seve	le Plants o ral Soybear	f Parents, Crosses	and	F1	and	$F_2$

Cross	Popula-	Plants	Linolenic Acid, %				Linoleic Acid, %				
Gross tion		tested	Range	Std. dev.ª	Mean	Std. error <sup>b</sup>	Range	Std. dev.	Mean	Std. error	
Grown	in field, 1954	·	-								
Henere Mid-est	TT	1		High x high	°		94.9.40.5	4.7	41.6	12	
Harosoy x Midwest	Harosoy	14	6.68-10.12	0.92	8.25	.24	04.4~40.0 95.0-40.1	3.8	42.0	1.5	
	Midwest	20	5.47-10.25	1.57	8.38	.35	33.2-49.1	5.0	272	2.2	
			5.44 - 9.08	1.32	7.54	.50	20 1 49 5	4.0	40.2	<u>.</u>	
	JT'2	102	5.09-10.87	1,33	7.90	.14	32.1-40.5	4.0	40.2	.*	
Milmut DI DI 07071	36:3			High x low			000 49 5	4 77	40.8	1 1	
Midwest x P.1.85671	Midwest	18	6.30-10.89	1.29	8.72	.30	33.2-48.3	94.7	40.0	9.4	
	P.1.85671	9	4.73 - 9.51	1.44	6.97	.48	33,1~00.1	1.0	91.0	95	
	11	2	6.83- 8.18	1.06	7.50	.75	29.2-34.0	3.3	01.0	4.5	
	F'2	22	4.82- 8.85		6.50	.20	28.2 - 48.0	5.5	34.4	• •	
		{	1	High x low			010 511	1.0	100	1 9	
Harosoy x P.1.85671	Harosoy	13	7.87-10.87	.85	9.06	.24	34.2~51.1	4.0	43.8	1.5	
	P.I.85671	7	5.87-6.78	.35	6.32	.14	35.1 - 43.0	2.4	39.8	.9	
	) F1	11	5.83-10.54	.89	7.50	.28	36.1 - 49.6	3.8	44.7	1.1	
	F2	57	4.17-10.33	1.41	7.54	.19	33.8 - 50.4	4.2	43.4	.0	
				Low x low				<b>a</b> <i>t</i>		0	
P.I.80476 x P.I.68423	P.I.80476	17	5.10-7.50	.60	6.13	.14	38.5 - 45.8	2.4	41.8	.6	
	P.I.68423	15	4.59-8.18	.77	6.22	.20	27.2 - 47.0	5.4	39.8	1.4	
	F1	2	6.43 - 6.44	.01	6.44	.01	35.0 - 36.8	1.3	35.9	.9	
	F <sub>2</sub>	29	3.47 - 7.97	.89	5.08	.17	29.0 - 45.8	4.4	38.2	.8	
	1			Low x low							
P.I.68423 x P.I.85671	P.I.68423	12	5.91-9.53	1.02	7.13	.30	32.5 - 44.4	3.4	37.7	1.0	
	P.I.85671	7	5.41 - 8.00	.99	6.54	.37	32.0-37.6	2.6	35.4	1.0	
	F1	5	3.49 - 5.25	57	4.45	.26	37.5 - 40.8	1.4	39.8	.6	
	F.	59	3.62 - 8.33	1 10	5.91	.14	31.2 - 53.5	3.9	39.4	.5	
	1	1	0.01	Low x low							
P.I.80476 x P.I.85671	PT 80476	16	5.52 - 7.01	35	6 28	.10	30.4 - 41.6	2.5	37.0	.6	
11100210111100011	P I 85671	Ĩŭ	4 95- 7 35	75	6.08	.24	34.3 - 45.2	4.0	38.5	1.4	
	F1	2	5 76- 7 39	106	6.58	75	35.2 - 37.8	1.8	36.6	1.2	
	F	65	341 - 701	61	4 84	10	28.8 - 46.0	3.9	38.9	.5	
	1 12	00	0.11 1.01	.01	1.0 4						
Grown in gr	eenhouse, 1958-5	9	1								
		····	-	Low x low		Į					
PI 80476 x PI 85671	P.L.80476	4	6 23- 7.67	71	6.75	.35	38.5 - 50.4	5.5	43.1	2.8	
11100110111100011	P.T.85671	4	4 56- 5 74	48	5 13	.24	27.5 - 35.5	3.5	31.2	1.8	
	F.	102	4 40- 8 32	91	5 99	10	26.6 - 52.9	6.3	38.5	.6	

<sup>a</sup> Standard deviation of single plants.
 <sup>b</sup> Standard error of mean.
 <sup>c</sup> Classification of high and low is arbitrary and refers to linolenic acid content only.

after isomerization in 15.5% potassium-t-butoxide in t-butanol at  $60^{\circ}$ C. for 20 hrs. (11) by a "bottle method" developed in this laboratory to facilitate the analysis of large numbers of fat samples. Occasionally the official method Cd 7-58 of the A.O.C.S. (12) was used in comparison with the other two techniques.

#### Results

Range of Percentage of Linolenic and Linoleic Acids. The amount of linolenic acid in oil from mature seeds of 251 soybean introductions grown in single plots ranged from 4.89 to 9.28% and linoleic acid from 35.8 to 53.4%. In 1,119 samples from single plants analyzed in the inheritance study, linolenic acid ranged from 3.35 to 11.0% and linoleic from 22.6 to 62.1%. The majority of seeds contained 18 to 22% of their dry weight as the lipid. There was no apparent correlation between fatty acid composition and lipid content.

Although each parent, especially when grown in the field, showed wide variations in oil composition from plant to plant (Table I), its average composition for the several years was fairly constant (Table II). Thus the two parents which were "high" in

linolenic acid were on the average slightly more than 2% higher than those selected as "low."

Field Studies of Progeny. With the exception of P.I.68423 x P.I.85671, in which a substantially lower amount of linolenic acid in the F1 than in either parent was evidence of heterosis, the  $F_1$  population was approximately intermediate between the parents in percentage of linolenic acid (Table I).

In  $F_2$  plants of all crosses the essentially continuous range of variation from small to large values indicated quantitative inheritance of linolenic acid. Environmental effects markedly influenced the quantity of this acid. The standard deviations of single plants were about as great for the parent and  $F_1$ as for the  $F_2$  populations (Table I). In Harosoy x P.I.85671 the 57 plants tested had a mean of 7.54% and ranged from 4.17 to 10.33%. Transgressive segregation to low amounts of linolenic acid in the F<sub>2</sub> population was observed, particularly in P.I.68423 x P.I.85671 and in P.I.80476 x P.I.85671.

Linoleic acid content also ranged widely in the progeny. Transgressive segregation to low values in F<sub>1</sub> and F<sub>2</sub> occurred in Midwest x P.I.85671. The greatest range in the amount of this acid in the F2

Mean Lino	lenic and L	inoleic Acid	Content o	f Soybean (	)il from Par	ents Grown	n in Differen	t Years		
1954—Field <sup>a</sup>			1955—Field <sup>b</sup>			1956—Field °		1958–59Greenhouse <sup>d</sup>		
Plants tested	Linoleic acid	Linoleic acid	Rows tested	Linoleic acid	Linoleic acid	Plants tested	Linolenic acid	Plants tested	Linolenic acid	Linoleic acid
No.	%	%	No.	74	S/r	No.	%	No.	%	%
38 27 32 33	8.56 8.67 6.49 6.21	41.3 42.2 39.7 39.4	5 6 8 3	8.84 8.47 7.10 6.57	$50.5 \\ 51.7 \\ 47.2 \\ 41.1 \\ 10.1$	 13 15	 5.60 6.05 6.92	1 12 4 4	9.52 8.65 5.08 6.69	$51.6 \\ 48.7 \\ 30.6 \\ 42.2$
	dean Lino 1 Plants tested No. 38 27 32 33 27	Mean         Linolenic         and         L           1954         Field         .	Mean Linolenic and Linoleic Acid           1954—Field *           Plants         Linoleic acid           rested         acid           No.         %           38         8.56           27         8.67           32         6.49           33         6.21           39.4         27	Mean Linolenic and Linoleic Acid Content o         1954—Field *         1           Plants tested         Linoleic acid         Linoleic acid         Rows tested           No.         %         %         No.           38         8.56         41.3         5           32         6.49         39.7         8           33         6.21         39.4         3           27         6.59         38.6         3	Image: Action of the second state in the se	Interpretation of the second content of Soybean Oil from Par           1954—Field *         1955—Field *           Plants         Linoleic         Linoleic         Rows         Linoleic         Linoleic         acid         acid         Acid         Acid         Acid         Acid         Inoleic         Linoleic         Linoleic         Acid         Acid <th< td=""><td>Interference of Source Content of Source Oil from Parents Grown1954—Field *1955—Field *1956—Plants testedLinoleic acidRows testedLinoleic acidLinoleic testedPlants testedNo.<math>%</math><math>%</math>No.<math>%</math><math>%</math>No.388.5641.358.8450.5278.6742.268.4751.7326.4939.787.1047.213336.2139.436.5741.115276.5938.636.2742.115</td><td>Interview of the text of text of</td><td>Image: Mean Linoletic and Linoletic Acid Content of Soybean Oil from Parents Grown in Different Years           1954—Field*         1955—Field*         1956—Field e         1958-           Plants tested         Linoletic acid         Rows tested         Linoletic acid         Plants tested         Inoletic acid         Plants tested         Inoletic acid         Plants tested         Inoletic acid         Plants tested         Plants tested         Inoletic acid         Inoletic acid         Inoletic acid         Inoletic acid         Inoletic acid         Inoletic acid         Inoletic acid</td><td>Idean Linolenic and Linoleic Acid Content of Soybean Oil from Parents Grown in Different Years1954—Field *1955—Field *1956—Field *1958–59—GreenhPlants testedLinoleic acidLinoleic testedLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidInoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleinic acidL</td></th<>	Interference of Source Content of Source Oil from Parents Grown1954—Field *1955—Field *1956—Plants testedLinoleic acidRows testedLinoleic acidLinoleic testedPlants testedNo. $%$ $%$ No. $%$ $%$ No.388.5641.358.8450.5278.6742.268.4751.7326.4939.787.1047.213336.2139.436.5741.115276.5938.636.2742.115	Interview of the text of	Image: Mean Linoletic and Linoletic Acid Content of Soybean Oil from Parents Grown in Different Years           1954—Field*         1955—Field*         1956—Field e         1958-           Plants tested         Linoletic acid         Rows tested         Linoletic acid         Plants tested         Inoletic acid         Plants tested         Inoletic acid         Plants tested         Inoletic acid         Plants tested         Plants tested         Inoletic acid         Inoletic acid         Inoletic acid         Inoletic acid         Inoletic acid         Inoletic acid         Inoletic acid	Idean Linolenic and Linoleic Acid Content of Soybean Oil from Parents Grown in Different Years1954—Field *1955—Field *1956—Field *1958–59—GreenhPlants testedLinoleic acidLinoleic testedLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidInoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleic acidLinoleinic acidL

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Planted: " May 19; b May 19; c June 15; d December 23.

P.I.68423.....

TABLE III									
Correlation Between F2 Plants and	F3 Progeny Rows for Linolenic and Lino Oil of Several Soybean Crosses	pleic Acid Percentage in Seed							

Cross Number of plants		Linolenic Acid, %					Linoleic Acid, %				Game
	F2 plants		F3 rov	F3 rows		F2 plan	F2 plants		F3 rows		
	anu rows	Range	Mean	Range	Mean	соеп.	Range	Mean	Range	Mean	coen.
Harosoy x Midwest Midwest x P.I.85671 Harosoy x P.I.85671 P.I.68423 x P.I.85671 P.I.80476 x P.I.85671	$ \begin{array}{r} 40 \\ 12 \\ 23 \\ 29 \\ 29 \end{array} $	5.09-10.87 4.82-8.38 4.69-10.33 4.16-8.33 3.82-7.01	7.766.447.796.04 $4.95$	$\begin{array}{c} 5.30{-}10.27\\ 6.32{-}8.88\\ 5.72{-}8.76\\ 3.77{-}7.63\\ 5.02{-}8.05\end{array}$	7.737.557.216.176.21	.433* .635 <sup>b</sup> .508 <sup>b</sup> .103° .055°	32.4-48.5 29.2-38.5 36.0-50.4 31.7-46.7 33.0-43.4	40.0 35.0 43.7 39.7 39.0	$\begin{array}{r} 35.6{-}62.1\\ 36.0{-}57.5\\ 31.2{-}48.6\\ 25.9{-}47.5\\ 31.6{-}47.2\end{array}$	$\begin{array}{r} 47.4 \\ 44.7 \\ 44.7 \\ 43.4 \\ 40.0 \end{array}$	026° 090° .495 <sup>b</sup> .213° .395 <sup>b</sup>
<sup>a</sup> Highly significant, 1	P<1%. » S	ignificant, P	between t	5% and 1%.	• Not sig	nificant, P>	>5%.				

population was from 31.2 to 53.5% in P.I.68423 x P.I.85671.

An attempt was made to find members from the  $F_2$  population of P.I.80476 x P.I.85671 which would yield a similar, or preferably a smaller, linolenic acid content in the  $F_3$  generation. Seeds from selected  $F_2$ plants were grown in 1955 and harvested as  $F_3$  rows. Table III shows that a highly significant relationship (P < 1%) between the percentage of linolenic acid in  $F_2$  and in  $F_3$  occurred only in Harosov x Midwest, in which both parents were "high." In Midwest x P.I.85671 and Harosoy x P.I.85671, each with high x low-linolenic-acid parents, the percentage of linolenic acid in the  $F_3$  population was significantly correlated (P between 5 and 1%) with that in the  $F_2$  selection from which it was derived. However, in P.I.68423 x P.I.85671 and P.I.80476 x P.I.85671, each between low-linolenic-acid content parents, there was a nonsignificant relationship between the  $F_2$  selection and the  $F_3$  generation.

The results for linoleic acid did not consistently correspond with those for linolenic acid. Harosov x P.I.85671 and P.I.80476 x P.I.85671 showed a significant positive correlation between the  $F_2$  plant selec-

tion and the resulting  $F_3$  population. In 1956 seeds from  $F_2$  plants containing "low" linolenic acid were planted, and the acid was ana-lyzed in the seed oil from individual  $F_3$  plants. Table IV shows that little correlation with respect to lowlinolenic-acid content was found between the F<sub>2</sub> plant selection and the  $F_3$  population derived from it. Only one of the 263  $F_3$  plants was as low as or lower than the  $\mathbf{F}_2$  selection in linolenic acid.

Greenhouse Studies. Data from an experiment in

	T in . I	Linolenic acid, %								
	acid, %	Plants tested	Range	Std. dev.	Mean	Std. error				
Parents grown in 1954			Parents grow	n in ī	956					
P.I.68423	6.59	15	4.91 - 8.28	0.97	6.92	.25				
P.I.80476	6.21	15	4.27 - 6.80	.62	6.06	.16				
P.I.85671	6.49	13	3.35 - 7.10	1.02	5.60	.28				
F2 plant selections grown in 1954		F3 populations grown in 1956								
CX269A-2 <sup>a</sup>	4.16	25	3.74 - 7.32	.90	612	.18				
-29	3.47	<b>24</b>	3.77 - 7.36	.67	5.77	.14				
CX270A-121	3.84	24	4.93 - 6.85	.50	5.86	.10				
C-49	3.62	<b>24</b>	5.65 - 8.46	.72	6.83	.15				
CX271A-11	3.41	21	5.88-9.14	.92	7.42	.20				
-22	3.87	25	5.31 - 7.57	.54	6.39	.11				
~115	3.82	25	5.31 - 7.95	.67	6.78	.13				
B-75	3.90	25	5.00 - 8.00	.90	6.48	.18				
-86	3.90	24	5.34 - 8.23	.66	6.65	.13				
U - 122	3.95	22	5.75 - 7.72	.56	6.52	.12				
-127	3.81	24	5.27 - 7.18	.64	6.24	.13				

TABLE IV F3 Progeny Tests of F2 Soybean Planta

CX269 (P.1.80476 x P.1.85671) CX270 (P.1.68423 x P.1.85671) CX271 (P.1.80476 x P.1.85671) A, B, and C refer to specific F1 plants from which F2 were grown.

1952 had shown no significant variability because of environment in the percentage of linolenic acid from 14 soybean varieties grown in four different field locations in Indiana with a north-to-south range of 150 miles. However a question arose as to whether environmental differences in separate years might partly explain why the  $F_3$  populations in 1956 with one exception had higher percentages of linolenic acid than the selections from  $F_2$  plants grown in 1954. Therefore a further investigation was made of Harosoy and an F<sub>2</sub> population of P.I.80476 x P.I.85671 grown in a greenhouse, in which environmental fluctuations were much less than those encountered in the field.



tents from F2 soybean plants of P.I.80476 x P.I.85671 grown in the greenhouse.

Harosov was used to measure variations in the amounts of linolenic and linoleic acids within a pure line variety. Seeds from 12 plants had a mean of 8.65% linolenic acid and a range of 7.57 to 9.60%and 48.7% linoleic acid with a range of 46.0 to 52.0%.

The distribution of F<sub>2</sub> plants of P.I.80476 x P.I.85671 in linolenic and in linoleic acid classes did not follow a similar pattern (Figure 1). The 102 plants grown in the greenhouse showed a frequency distribution skewed from a single peak for linolenic acid with the greatest number of plants in the 5.00 to 5.49% class. Only about 10% of the population contained less than 5% linolenic acid in the seed oil.

The frequency distribution for linoleic acid showed more than one peak. Values from 22.6 to 52.9% emphasized the wide spread of linoleic acid percentages in the  $F_2$  generation. The lowest amounts of linoleic acid detected in this study came from plants grown in the greenhouse. Four plants produced seeds containing less than 25% linoleic acid in the oil.

Relation Between Linolenic and Linoleic Acids. A graph of linolenic against linoleic acid content of soybean oil showed a highly significant correlation for the plants grown in the greenhouse. This was true for Harosoy (r = .96; P < 1%) (Figure 2-A) and for the  $F_2$  population of P.I.80476 x P.I.85671 (r = .89; P < 1%) (Figure 2-C).

A highly significant correlation (r = .75; P < 1%)between the proportions of these two acids was also found for Harosoy plants grown in the field in 1954 (Figure 2-B). The relationship was not as close with the field- as with the greenhouse-grown plants.

Conversely a very low correlation between the linolenic and linoleic acid percentages from field-grown plants was found for the  $F_2$  population of P.I.80476 x P.I.85671 in 1954 (r = .20; P > 5%) (Figure 2-D) andfor the F<sub>3</sub> generation in 1955 (r = .13; P > 5%) (Figure 2-E).

#### Discussion

The oil from 1,370 samples of mature soybean seeds analyzed in this study contained 3.35 to 11.0% linolenic acid and 22.6 to 62.1% linoleic acid. Obtaining seeds from cultivated varieties of Glycine max. grown under similar environmental conditions with a content of acids outside these ranges is not likely for two reasons. a) The 251 plant introductions showed wide variation in vegetative growth, maturity, flower and pod color, seed shape, color, weight, and oil and protein contents and thus represent a reasonable sampling of soybean varieties. b) The crossing in all possible combinations of the three-plant introductions containing the least linolenic acid did not yield any F2 plants with a substantial decrease in the amount of this acid, which remained equally low in the  $F_3$  population (Tables III and IV). Perhaps more plants should be grown and the seeds analyzed to detect any which may be homozygous for low linolenic acid content, particularly if a large number of interacting genes influence this characteristic. It is possible that other combinations of low x low parents might be more effective in giving transgressively lower values.

Values outside the range of 3.35 to 11% linolenic acid in soybean oil were observed with the thiocyanogen method of analysis (13,14), but they have not been reported since the advent of the spectrophotometric determination of fat composition, with two exceptions. a) Mature seeds from plants grown in controlled climate chambers under conditions which were abnormal to those encountered in the field yielded a value of 16.1% (8); and b) assays of the oil from immature beans of the Lincoln variety, which showed 23.4% of linolenic acid, at seed maturity showed that the proportion of this acid had decreased to 6.2% (15).

Percentages of linoleic acid in soybean oil below 40% or above 60% are not often found in the literature published since 1941. with the older analytical techniques which are also known to give low measurements of linoleic acid (16), only one value below 30% was found in 95 samples with iodine values ranging from 99.6 to 147.6 (3). This same report listed 64.8% linoleic and 10.1% linolenic acid in the wild soybean (Glycine ussuriensis). As far as the authors know, the four F<sub>2</sub> samples of P.I.80476 x P.I.85671 grown in the greenhouse with a content of linoleic acid between 22.5 and 25.0% (Table I) show the smallest proportion of this acid ever detected in soybean oil.

Environment as well as variety had considerable effect on polyunsaturated fatty acid composition. While Midwest and Harosoy varieties consistently

55 35 45 50 20 25 30 40 LINOLEIC ACID - % 2 - D 2 - E r = .20 = .13 8 6 5 3 30 35 45 35 45 40 40 LINOLEIC ACID - % FIG. 2. Regression of linolenic on linoleic acid contents of oil from mature soybean seeds. (A) Harosoy plants grown in the greenhouse and (B) field; (C)  $F_2$  population of P.I.80476 x P.I.85671 grown in the greenhouse and (D) field; (E) Fs population of P.I.80476 x P.I.85671 grown in the field. The corre-

produced oils with a higher mean proportion of linolenic and linoleic acids than the three plant introductions selected for breeding purposes, variations between plants were wide. The controlled environment of the greenhouse decreased the ranges of variation between plants but did not affect the spread between the varieties. P.I.85671 exercised less control over the mean percentage of linolenic acid in the different growing seasons than did the other four varieties in which this value ranged within one-percentage unit (Table II). Similarly the mean linoleic acid content of this parent as compared with the others was more variable.

lation coefficients are shown in the upper left corners.



Inheritance of linolenic and linoleic acids in soybean seeds appeared to be quantitative rather than qualitative (Table I). Because of the lack of parents differing widely in linolenic and linoleic acid contents and the consequent overlapping in acid percentage of some members, it was not possible to estimate the number of gene pairs exerting a cumulative effect on the production of the two acids. Harosoy x P.I.85671 exhibited well the blending of characteristics of the two parents in the  $F_1$  generation, followed by the complete range of variation in the  $F_2$  population from the one parental series of values to the other. In some instances, plants that extended beyond the parental extremes and indicated transgressive segregation were obtained.

Some evidence was found for a dissimilarity in the manner of inheritance of the two acids, particularly in F<sub>2</sub> plants of P.I.80476 x P.I. 85671. The frequency distribution pattern for plants grown in the greenhouse was different for the two acids (Figure 1). Moreover plants grown in the field showed a considerable amount of transgressive segregation to low percentages of linolenic acid. This was not true for linoleic acid (Table I).

The lack of completely effective selection in  $F_2$  to obtain F<sub>3</sub> plants with low amounts of linolenic acid (Table IV) was probably due to environment. The high variability among single plants in these data show the need for replicated plots when evaluating for linolenic and linoleic acids.

Comparison of field and greenhouse data from  $F_{2}$ plants of P.I.80476 x P.I.85671 demonstrated the presence of an influential factor in the field which destroyed the close correlation (P < 1%) between the amounts of linolenic and linoleic acids found in seeds grown in the greenhouse (Figures 2-C and 2-D). The  $F_2$  plants in both instances came from the same  $F_1$ population.

The nearly perfect correlation between the two acids in Harosoy from the greenhouse was decidedly lessened in the field environment (Figures 2-A and 2-B). The regression coefficient of linoleic on linolenic acid for Harosoy was 3.3 whereas that for  $F_2$  members of P.I.80476 x P.I.85671 was 7.2; both values are for plants grown in the greenhouse. Thus, if any kind of equilibrium between the two acids was operative through their interconversion, an increase in one relative to the other was not the same in different populations. However the high correlation between the two acids clearly showed their association rather than their independence during the fat-deposition period.

Barker and Hilditch (17) concluded that the more unsaturated oils from sunflower seeds were a result of slow development and ripening of the seeds and consequent slow fat production. They considered temperature and probably the amount of direct sunlight incident on the ripening seed heads as the most important factors controlling the rate of seed development and hence the composition of the fat. A similar inverse effect of temperature on fat unsaturation was noted for soybean seeds by Howell and Collins (8). They also found a positive correlation between the proportions of linolenic and linoleic acids (6). It may be that the light intensity or quality reaching the developing sovbean seeds and temperature effects in the present work was responsible for the close correlation between the acids in seeds from the greenhouse in contrast to those from the field.

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# Safflower Oil Adducts as Plasticizers 1.2

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Eleven esters and epoxides of adducts of conjugated linoleic acid with maleic and acrylic acids, and eight esters and epoxides of adducts of vegetable oils with acrylic and maleic esters

were evaluated as plasticizers for polyvinyl chloride (PVC), acrylonitrile rubber, and polyvinylidene chloride, and in PVC plastisols. The dimethyl ester of the acrylic adduct of linoleic acid and its epoxide were the most promising as plasticizers for PVC and in PVC plastisols as their performance compared favorably with that of controls. In polyvinylidene chloride however these adducts had a slight adverse effect on color stability. The vegetable oil adduct esters and epoxides were incompatible with PVC but had extremely good compatibility with acrylonitrile rubber. In general, they performed like the

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