Fatty Acid Accumulation in Maturing Flaxseeds as Influenced by Environment[1]

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Summary. The effects of temperature and light on boll and shoot maturity and on the accumulation of fatty acids in developing seeds of flax (*Linum usitatissimum* L.) were determined in controlled environments. Palmitic and linoleic acids decreased but linolenic increased in percent as seed formation progressed. In the same period, oleic acid increased in percent in 1 variety and decreased in another. Increased temperatures hastened these changes and resulted in decreased iodine value of the oil at maturity.

Calculated on a weight basis (mg per 1000 seeds), all 5 major fatty acids increased during seed formation. Increased temperatures initially accelerated the accumulation of all fatty acids, but the period of net fatty acid synthesis was eventually shortened in comparison with cooler temperatures. At 15° and 20°, linolenic acid accumulation closely paralleled the rate of boll maturation, measured by boll moisture content; at 30° linolenic accumulation ceased before maturation could be detected.

A photoperiod of 20 hours accelerated plant maturity resulting in decreased seed weight in comparison with a photoperiod of 16 hours. Eight hour photoperiod favored late blossoming and depressed seed weight, oil content, and fatty acid content. Weights of linoleic and linolenic acids were high in both the 16 and 20 hour photoperiods.

Linolenic acid was reduced in percent and weight per 1000 seeds at light intensities of 1200 ft-c as compared with 2700 ft-c.

The fatty acid composition of reserve fats deposited in seeds is markedly influenced by environment (16). For flax, the environmental factor of primary importance appears to be temperature (2, 4, 7, 8), but other environmental factors may also be involved (6, 8, 21). The mechanisms by which environmental effects are produced have not been clearly defined.

In the work reported here, the technique of observing the changes in fatty acid levels which occur in developing seeds during the period from fertilization to maturity was used to assess the effects of temperature, photoperiod, and light intensity on fatty acid biosynthesis in flax. Studies by this technique provided early insights into fatty acid biosynthesis in oilseeds (14, 23), and the method still is commonly employed for this purpose since fatty acid composition can readily be determined by gas chromatography (1, 12, 13, 20). Responses to environment other than changes in fatty acids were also measured in the present studies. Rate of maturation was of special interest since it is clearly affected by environment (7, 18) and may have especially significant consequences on oil formation (11).

Materials and Methods

Most of the techniques used in these studies have been described earlier (7). Temperature, photoperiod, and light intensity were regulated by plant growth chamber equipment of 2 types. Cool-white fluorescent and incandescent lamps provided light intensities, measured with a Weston[2] Model 756 light meter, of 1200 ft-c and 2700 ft-c in the 2 chambers (5.1 and 7.1 mw/cm² respectively measured with an Eppley Laboratory thermopile with quartz window). Temperature control was ± 1.5° measured at boll height. Humidity was uncon-

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[2] Mention of proprietary products herein does not necessarily imply indorsement of these products by the United States Department of Agriculture.

trolled. The plants were grown 5 per pot in 1-liter plastic containers of aerated nutrient solution or 10 to 15 per pot in 2-liter glazed crocks containing vermiculite watered with nutrient solution. Pots were completely randomized in the chambers with 3 to 4 replications harvested at each date of harvest. Conditions of 20° and 16 hour photoperiod per 24-hour cycle were maintained during the prebloom stage in all experiments. Test environments were started only after the initiation of flowering.

Environments tested during the flowering stage in the 3 experiments were (1) day temperature study; 15°, 20°, 25°, and 30° day temperatures, night temperature 20°, light intensity 2700 ft-c, and photoperiod 16 hours; (2) photoperiod study; 8, 16, and 20 hour photoperiods, 20° constant temperature, and light intensity 1200 ft-c; (3) light intensity study; 1200 and 2700 ft-c light intensities, 20° constant temperature, and 16 hour photoperiod. Environment treatments within an experiment usually were not repeated in other chambers. However, good agreement has been obtained in experiments repeated several times in 1 environment and in several chambers. This is indicated by a coefficient of variation of 3.7 % for iodine value determinations from a total of 33 replications in 4 separate trials at 20°.

Plants were harvested from the test environments at several dates during the period of boll development. Characteristics measured included plant height and weight, boll and seed production, and oil content and quality. Seed samples analyzed for oil characteristics were limited in age by harvesting bolls previously tagged with colored strings during a 3 to 4 day period at initiation of blossoming. The tagged bolls were excised, oven dried at 100°, and threshed. Seed weight per 1000 seeds was determined from the weight of a 150-seed sample, and oil percent was determined by micro-Soxhlet extraction of the crushed seeds with petroleum ether (bp 30–60°). Methyl esters of the long-chain acids were prepared by transesterification of a portion of the extracted oil with sodium methoxide (3). The esters were analyzed with an Aerograph A-90-P2 gas chromatograph employing a 3m × 6.4mm ID column of 20 % (w/w) diethylene glycol succinate on 60 to 80 mesh Gas Chrom P (Applied Science Company) at 205°. Peak areas on the recorder chart were determined by an attached integrator (Disc Instruments, Incorporated). Weight percentages of the fatty acids were determined from area measurements by calibration with pure fatty acid esters (The Hormel Institute, University of Minnesota). Weights of fatty acids per 1000 seeds were calculated from seed weight, oil percent, and fatty acid composition assuming 100 % triglyceride composition of the oil. Theoretical iodine values were calculated from fatty acid composition.

The varieties used in these studies were C.I. 2224, and C.I. 1666, and C.I. 1303 and represented respectively the high, intermediate, and low limits of linolenic acid composition presently available in lines of *Linum usitatissimum* L. Typical curves depicting the changing unsaturation in the oils of maturing seeds of these varieties when grown in the field environment are presented in figure 1.

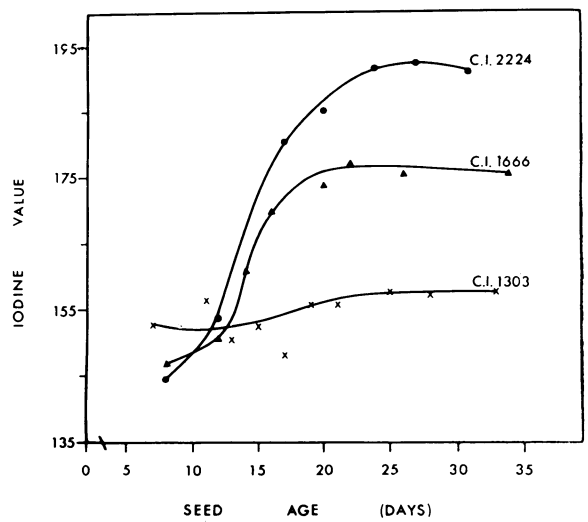

FIG. 1. Progressive changes during maturation under field conditions in the iodine value of seed oils of the 3 flax varieties used in controlled environment studies. Grown at the South Dakota Agricultural Experiment Station in 1963 and 1964.

Results

Temperature Effects on Fatty Acid Accumulation. Since temperature is customarily assigned a major role in regulation of fatty acid composition of oilseeds, this environmental factor was the first studied. Plants of C.I. 1303 and C.I. 2224 were grown from flowering to maturity at day temperatures of 15°, 20°, 25°, and 30°. Measurements made at boll maturity showed that increased temperatures reduced plant weight, moisture percent, seed weight, oil content, and iodine value of the oil (table I). Relative quantities of the fatty acids during the period from 9 days after flowering to maturity were markedly affected by temperature (table II). Palmitic and linoleic acids decreased in percent as the seeds matured, while linolenic increased. Increased temperatures hastened but did not alter the direction of these changes. Oleic acid percent was markedly increased by warm temperature, but the change in percentage of this acid from flowering to maturity depended upon the variety tested. In the low iodine value variety C.I. 1303, oleic acid increased in percentage, while in C.I. 2224, oleic acid decreased with advancing maturity (fig 2).

Calculated on weight basis, all 5 fatty acids increased in weight per 1000 seeds as seed develop-

Table I. *Influence of Temperature on Flax Growth and Seed Production*
The variety was C.I. 1303, light intensity 2700 ft-c, photoperiod 16 hours, and night temperature 20°.

Day temperature	Plant dry wt g/10 Plants	Shoot moisture %	Seeds Per Boll	Seed Wt mg/1000 Seeds	Oil content %	Iodine value
15°	22.4a*	44a	6.8a	6110a	38.2a	176a
20°	19.6a	45a	8.3a	5770b	37.8a	164b
25°	14.5b	26b	7.0a	4910c	31.9b	157c
30°	13.8b	17b	6.9a	4360d	31.4b	140d

* Adjacent values within a column having a common letter do not differ significantly at the 5 % level.

Table II. *Changes in Fatty Acid Composition of Maturing Seeds of C.I. 1303 Flax as Influenced by Temperature*

Temperature	Seed age, days	Fatty acid composition (% of total fatty acids) Palmitic	Oleic	Linoleic	Linolenic
15°	9	13.2a*	23.2a	19.5a	41.7a
	16	11.8b	23.5a	21.3a	41.0a
	23	8.1c	27.0b	14.1b	48.5b
	37	6.5d	28.0b	14.6b	48.7b
20°	9	13.6a	24.9a	20.6a	38.6a
	16	11.0b	29.7b	17.9b	39.0a
	23	7.6c	35.2c	10.5c	43.9b
	37	6.8d	35.7c	11.5c	43.7b
25°	9	13.9a	27.6a	20.1a	36.3a
	16	10.0b	35.2b	12.9b	39.6b
	23	7.8c	37.7b	10.1c	42.2b
	37	7.6c	39.9b	9.9c	40.5b
30°	9	13.1a	41.8a	17.0a	25.5a
	16	9.0b	46.6b	11.3b	30.4b
	23	8.3c	47.1b	11.0b	31.0b
	37	8.3c	47.4b	10.6b	30.9b

* When values for individual fatty acids at the 4 seed ages within a temperature group are arranged in order of magnitude, adjacent means followed by a common letter do not differ significantly at the 5 % level. Stearic acid content averaged 2.4 % and was unaffected by temperature and age.

Table III. *Daily Increase in Fatty Acids in C.I. 1303 Flax Seeds as Influenced by Temperature and Seed Age.*

Temperature	Seed age in days 0–9	10–16	17–23	24–37
	mg of fatty acids per 1000 seeds per day			
Linolenic Acid:				
15°	6	26	74	24
20°	4	37	76	0
25°	9	40	39	0
30°	9	31	15	0
Linoleic Acid:				
15°	3	13	15	8
20°	2	17	9	3
25°	5	10	5	0
30°	6	8	5	0
Oleic Acid:				
15°	3	15	41	15
20°	3	29	63	5
25°	7	38	35	0
30°	15	46	23	0
Saturated Acids:				
15°	2	8	12	2
20°	2	12	14	0
25°	4	11	5	0
30°	6	9	4	0

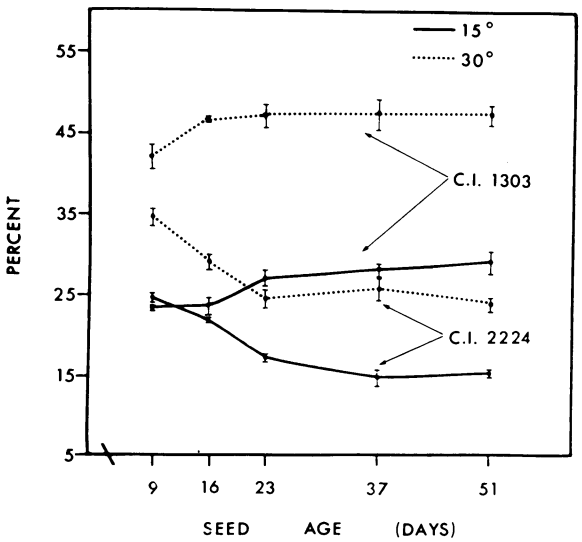

FIG. 2. Changes in oleic acid percentages of 2 flax varieties during seed development as influenced by temperature. Vertical lines indicate standard error of the mean.

ment progressed. Palmitic, stearic, and linoleic acids accumulated slowly, while oleic and linolenic acids accumulated rapidly (table III). Both the rate and period of accumulation of the acids were altered by temperature with increased temperature initially hastening fatty acid accumulation in very young seeds but later shortening the period of acid increase. After 23 days of age, accumulation of the fatty acids was observed only at the cooler temperatures of 15° and 20°. Daily rates of accumulation of linolenic acid were greater in seeds of the high iodine value variety C.I. 2224 (reaching a max of 143 mg per 1000 seeds per day at 20°) than in C.I. 1303.

Since warm temperatures shortened the period of accumulation of linolenic acid, the rate of boll maturation was evaluated from moisture content data. Boll moisture percentages for the variety C.I. 1303 declined slowly and uniformly at all temperatures during the first 23 days after flowering (fig 3). At 20° and above, the bolls matured during the period from 23 to 37 days, and moisture content declined sharply during this period. At 15°, however, maturity was retarded, and boll moisture content remained relatively high at 37 days. Rates of accumulation of linolenic acid closely paralleled moisture content changes at 15° and 20° but not at 30° where net linolenic acid synthesis declined before the bolls matured.

Photoperiod and Light Intensity Effects on Fatty Acid Accumulation. In the photoperiod study, C.I. 1303 flax was grown to flowering at a constant temperature of 20° light intensity of 1200 ft-c, and photoperiod of 16 hours. Eight days after flowering, the plants were transferred to photoperiods of 8, 16, or 20 hours per 24-hour cycle. The 20 hour photoperiod markedly hastened shoot senescence in comparison with the other treatments. Within 5 weeks after flowering, plants subjected to 20 hour photoperiods had matured and all tissues were desiccated. In the 16 hour and 8 hour photoperiods the earliest bolls had matured by the fifth week, but plant growth was active and, in the case of the shorter light period, many new blossoms were being produced. These visual observations correlated with photoperiod effects on seed and fatty

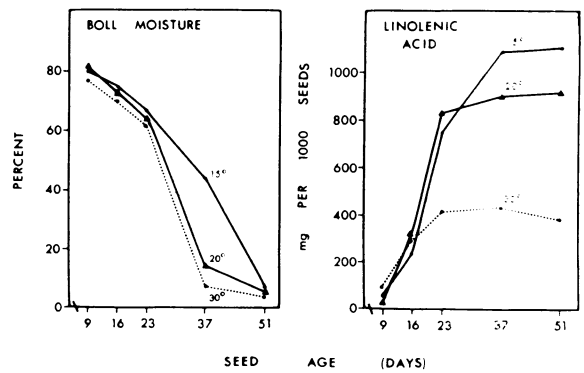

FIG. 3. Rate of boll maturation compared with accumulation of linolenic acid in maturing flaxseeds.

Table IV. *Influence of Photoperiod on Seed and Oil Characteristics of Flax*

The variety was C.I. 1303, temperature 20°, and light intensity 1200 ft-c.

Characteristic measured	Photoperiod (hrs) 8	16	20
Seed wt (mg/1000)	4490	6170	5310
Oil content (%)	32.3	36.4	38.0
Iodine value	173	164	174
Fatty acids (mg/1000):			
Palmitic	98	145	130
Stearic	22	43	36
Oleic	444	816	562
Linoleic	148	195	262
Linolenic	677	960	928

acid weights. Maximum seed weight was observed in the 16 hour photoperiod (table IV). Early senescence in the 20 hour photoperiod was accompanied by a slight reduction in seed weight in comparison with 16 hours; fatty acid weights also decreased except for linoleic and linolenic acids. The very active late blossoming observed in the 8 hour photoperiod was accompanied by marked reductions in seed weight, oil content, and fatty acid content of the first formed seeds.

Finally, when both temperature and photoperiod were held constant (20° and 16 hours respectively) while light intensity was varied, fatty acid com-

Table V. *Influence of Light Intensity on Seed and Oil Characteristics of Flax*

The varieties were C.I. 1303 and C.I. 1666, temperature 20°, and photoperiod 16 hours. Three experiments with 16 total observations were conducted at 1200 ft-c and 6 experiments with 60 observations at 2700 ft-c.

Characteristic measured	Light intensity (ft-c) 1200	2700	Statistical analysis
Seed wt (mg/1000 seeds)	5700	5850	NS
Oil content (%)	36.6	40.1	NS
Iodine value	162	181	Significant (1 %)
Linolenic acid (%)	43.6	52.8	Significant (1 %)
Linolenic acid (mg/1000 seeds)	870	1190	Significant (1 %)

position of the oil in flaxseeds was again altered. Repeated trials were conducted with the varieties C.I. 1303 and C.I. 1666 in controlled environment chambers differing in light intensity. Iodine value and linolenic acid content of the oil were significantly lower at 1200 than at 2700 ft-c (table V). Seed weight and oil content did not differ significantly in the 2 light regimes.

Discussion

Temperature, photoperiod, and light intensity all affected oil formation in flax when tested under controlled conditions permitting study of isolated environmental stresses. The effects on oil quality were accompanied by other plant responses, especially changes in rate of maturation. The environmental influence on boll maturity sometimes paralleled the effect on fatty acid composition, but at other times the 2 effects were not concurrent. These and other findings from the present study are relevant to questions of fatty acid biosynthesis and mechanism of environmental effects on oil formation.

When early workers found that linolenic acid increased in percent in maturing flaxseeds while oleic and linoleic decreased, they concluded that oleic was progressively desaturated into linoleic and finally into linolenic acid (14, 16, 23). The interpretation was challenged because all fatty acids actually increase in weight during maturation without any indication of interconversion between acids (9, 11, 18). In the present study palmitic and linoleic acids did decline in percent with advancing maturity, while linolenic increased. However, there was no consistent oleic-linolenic relationship, since in 1 variety oleic acid content declined during maturation while in a second variety oleic increased. Thus, where percentage data are concerned, success in demonstrating apparent conversion of oleic to linolenic in flax depends upon the variety investigated.

Recent studies by radioactive tracer techniques have demonstrated in vivo time-changes in fatty acid labeling patterns in flax similar to the changes in fatty acid percentages occurring during the maturation of a high-linolenic variety (5). Similar findings have also been reported from studies with soybean seed (19) and castor bean leaves (10). These findings coupled with evidence obtained with cell-free systems (15, 22) strongly favor desaturation of oleic acid as the pathway of biosynthesis of long-chain polyunsaturated fatty acids. Interpreting the effect of temperature on fatty acid composition in this light, it would appear that the activity of a desaturating system was initially enhanced by warm temperatures. Ultimately, however, the activity of the system was depressed resulting in reduced levels of polyunsaturated acids in the mature seeds grown in warm temperatures. The finding by Meyer and Bloch (17) that the activity of a desaturating system from *Torulopsis utilis* was greater when the cells had been grown at a cool temperature than at a warm temperature supports this view. On the other hand, Hilditch (11) argued that the polyunsaturated 18-carbon acids are not formed by desaturation of oleic acid and offered as evidence the response to temperature. Warm conditions, he argued, should hasten fat synthesis and thereby increase the level of the acid which terminates the interconversion sequence. This should result in increased rather than decreased levels of linolenic acid at warm temperatures if a desaturation mechanism is involved. In the present study, we observed that increased temperatures did, for a time, accelerate the accumulation of linoleic and linolenic acids as well as oleic and the saturated acids. Measurements of fatty acid composition made only at seed maturity would not have revealed the rate-enhancing effect of warm temperatures on polyunsaturated acid accumulation, however, because of the simultaneous shortening of the period of acid accumulation which decreased acid weights in the mature seeds.

Thus, maturity is a factor which appears closely related to environmentally induced changes in fatty acid composition of flax in some circumstances. Fatty acid composition at 15° and 20° closely paralleled rates of boll maturation at the 2 temperatures with the more slowly maturing bolls (15°) also attaining the higher level of linolenic acid. However, the high temperature-induced reductions in polyunsaturated acids were not due solely to the shortened period of fatty acid accumulation since

at 30° the net synthesis of linolenic declined more than a week before boll maturation could be detected. Moreover, long photoperiods markedly hastened senescence of the flax plants, and yet the levels of polyunsaturated acids produced were high both in percent and weight per 1000 seeds. The response of the developing flaxseed to environmental stresses appears to be complex and must involve effects on fatty acid biosynthesis per se as well as interrelated effects on other plant processes such as flowering and maturity.

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