Changes in Fatty Acid Composition of Sunflower (*Helianthus annuus*) Seeds in Response to Time of Nitrogen Application, Supply Rates and Defoliation*

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ABSTRACT

The fatty acid composition of sunflower (Helianthus annuus L) *seed oil was
shown to respond to N supply rates and the time of N application in four
glasshouse and two field experiments using five cultivars. Changes in
individual fatty acids* % *total were smaller than those caused by differences in
minimum temperatures during seed development but they were statistically
significant. N supply rates before floret initiation affected the* % *fatty acid
composition of mature seeds: the percentage of palmitic (16:0) and linoleic
(18:2) acids responded positively to increases in N supply whereas* % *stearic
(18:0) and oleic (18:1) acids responded negatively. The only fatty acid to
respond to N supply rates between floret initiation and anthesis was 18:0
acid. The percentage of 18:1 responded positively and that of 18:2 acid
negatively to high N supply rates after anthesis. Responses to single N
applications at different stages of plant development in the field confirmed
these findings but results were more variable than in the glasshouse. Partial or
complete defoliation at anthesis in the field caused the same changes in fatty
acid composition as did a low N supply after anthesis.*

The results are difficult to interpret in terms of our current knowledge of lipid biosynthesis. N supply rates may affect the rate of hydrolysis of fatty acid complexes or their transport from the proplastid to the cytosolic compartment. There was no compelling support for the hypothesis that N supply rates before floret initiation were affecting fatty acid composition through their effects on other plant characters such as leaf mass, seed number or single seed weight.

Key words: Sunflower seed, nitrogen supply, defoliation, oil quality, fatty acids.

INTRODUCTION

During the development of sunflower (*Helianthus annuus* L) seed the proportion of oleic (18:1) acid in the oil decreases and linoleic (18:2) acid increases (Simpson and Radford 1976; Robertson *et al* 1978). The fatty acid composition is also known to differ between cultivars and with environmental conditions. A number of reports (Canvin 1965; Keefer *et al* 1976; Goyne *et al* 1979; Unger 1980) have elicited a major role for temperature in fatty acid composition. Night temperature during the time of seed lipid synthesis has been shown to have the largest effect (Harris *et al* 1978; Rochester and Silver 1983; Seiler 1986): the higher the temperature, the lower is the level of 18:2 and the higher the proportion of 18:1. This has also been demonstrated in cultured isolated sunflower embryos (Silver *et al* 1984), suggesting that temperature has a direct effect on seed metabolism.

By contrast with temperature, very little has been published on the effect that N supply rates have on the fatty acid composition of sunflower oil. Djemal *et al* (1985) reported that % 18:1 acid is less and % 18:2 acid more in N-sufficient plants compared with N-deficient plants. This contrasts with flax (Dybing 1964) where an increase in N supply decreased % 18:2 while increasing % 18:1 acid. The latter changes are related to a decrease in the iodine value of the oil, and such a decrease has been reported for flax and safflower (Yermanos *et al* 1964) occasioned by the application of N. Thus species appear to differ in their response to N.

The response of sunflower to N depends upon the time of its application, especially for components of seed yield. Thus seed number per plant responds positively to N supply before floret initiation (Steer and Hocking 1983; Steer *et al* 1984) through its effect on the number of florets initiated (Palmer and Steer 1985). Single seed weight responds positively to N application after floret initiation. Application after anthesis decreases the oil concentration (mg g^{-1} dry weight) and increases the N concentration (mg g^{-1} dry weight) in seeds, especially by increasing the content of some free amino acids (Steer *et al* 1984).

The nitrogen available to sunflower can vary from crop to crop, and N fertiliser can be and is applied at different stages of crop development (Steer and Hocking 1985). The quality of sunflower seed oil (ie the relative proportions of fatty acid residues) is an important attribute of yield particularly for marketing and processing. It is therefore important to know how oil quality is affected by the N

status of the plant. This paper reports the response of fatty acid composition to the timing and rate of N application.

MATERIALS AND METHODS

The results from four glasshouse experiments (1–4) and five field experiments (5–8) carried out in Griffith, New South Wales, are reported. In most cases experimental procedures have been published previously and they are listed in Table 1 along with the objectives of each experiment and the cultivars used. The hybrid Hysun 30 and the open-pollinated line, Sunfola 68·3, were obtained from Pacific Seed Co.* The Sirosun cultivars were from an experimental breeding program at CSIRO, Griffith, New South Wales. Sirosun 132H and 152H were F1 hybrids. Sirosun 892952 was from a three-way cross. Sirosun Bianca Grande was an open-pollinated confectionary line, and Siroleo was an early-maturing oilseed selection from Peredovik.

Glasshouse experiments

The four glasshouse experiments, including the previously unpublished experiments 2 and 3, were conducted in a naturally lit house. Seeds were sown in 8-litre pots of washed, screened sand, and plants were thinned to one per pot at the two-leaf stage. Plants received water until the cotyledons had expanded, and thereafter 250 ml per plant of a complete nutrient solution (see Steer and Hocking 1983 for composition) was supplied each morning and water as required at other times of day. Every 10 days and when the N supply rates were changed, the pots were flushed with water to remove accumulated salts. Other mineral elements were supplied at adequate rates but the N supply ranged from severely inadequate (1·7), inadequate (8·7), adequate (21) and luxury amounts (42 mg N d^{-1} per plant) when judged by the response of plant size, mass and seed numbers (Steer and Hocking 1983). The different N supplies were achieved by varying the nitrate concentrations in the nutrient solutions.

N supply rates were changed at the completion of floret initiation (FI) in experiments 1–3. Plants from each supply rate before FI (1·7, 8·7, 21 and 42 mg N d^{-1} per plant) were changed to one of the four supply rates after FI in experiment 1 (at 44 days after sowing: DAS) so that there was a 4 × 4 matrix of treatments of N supply rate before × supply rate after FI. This allowed the effects of N supply rates before and after FI to be tested on the fatty acid composition of mature seeds. In experiment 2, N supply rates (1·7, 8·7 and 42 mg N d^{-1} per plant) were changed at 57 DAS (FI) so that there was a 3 × 3 matrix of treatments. The same N supply rates were used in experiment 3 and they were changed at 40 DAS, which was also at the end of FI. In experiment 4, two supply rates (8·7 and 42 mg N d^{-1} per plant) were used. Plants had their N supply rates changed at three-row anthesis (59 DAS). In this experiment the effect of N supply rates before and after three-row anthesis could be analysed in a 2 × 2 matrix.

* Mention of a trade name or product does not constitute a recommendation or endorsement for use by the US Department of Agriculture, nor does it imply registration under FIFRA as amended.

TABLE 1

The objectives and location of the experiments, the cultivars used, and the references containing complete procedures for each experiment

Objective	Experiment	Cultivars	Location	References
To test the effect of N supply rates before and after F1 on seed characters	1	Hysun 30	Glasshouse	Steer and Hocking (1983) Steer et al (1984) Expt 1
To test N supply rates before and after 3-row anthesis on seed characters	2	Sirosun 892952	Glasshouse	See text
	3	Sunfola 68:3	Glasshouse	See text
	4	Hysun 30	Glasshouse	Steer et al (1984) Expt 3
To determine effects of timing of N application on seed characters	5	Hysun 30 Sirosun 132H Sirosun Bianca Grande	Field	Steer and Hocking (1985) Expt 1 Steer and Hocking (1985) Expt 3
	6	Sunfola 68:3 Sirosun 892952	Field	Steer and Hocking (1987) Expt 2
To determine the effect of defoliation at anthesis on seed characters	7	Sirosun Bianca Grande	Field	Steer et al (1988) Expt 3
To test differences between cultivars on seed characters	8	Sirosun Bianca Grande Sirosun 152H Hysun 30 Siroleo	Field	Steer and Hocking (1987) Expt 1

Plants were harvested at physiological maturity: at 103 DAS in experiment 1, 144 DAS in experiment 2, 118 DAS in experiment 3, and 125 DAS in experiment 4.

In the glasshouse, replicate pots were grouped into north and south blocks, and plots of inside and outside rows on the trestle, in order to identify any position effects. Analyses for the effect of N supply before and after the completion of floret initiation were made on the values per plant. The results from experiments 2 to 4 were analysed by analyses of variance and those from experiment 1 by regression analyses. Means were separated at $P = 0.05$ by Duncan's Multiple Range Test.

Field experiments

The field experiments were conducted on a transitional red–brown earth. Irrigation water was metered on to each treatment plot $(11 \times 12$ m) through a machine that also allowed measured amounts of urea to be applied in the water. The necessity for irrigation was calculated from pan evaporation and tensiometer readings, and N application from the stage of crop development. Plant population densities were 100 000 plants ha^{-1} in experiments 5 and 6 and 75 000 in experiments 7 and 8. In experiment 5, 120 kg N ha^{-1} was applied at different stages of crop development (see Fig 1). In experiment 6, 100 or 200 kg N ha^{-1} was split between 29 and 65 DAS. In experiments 7 and 8, 50 kg N ha^{-1} was applied three times: before sowing, at FI and at early anthesis.

Experiments 5, 6 and 8 were of a split-plot design with blocks (5, 4 and 3 respectively) containing one of each treatment plot. The plots were split for the cultivars used (see Table 1). Analyses of variance were made on the plot means of six plants at physiological maturity.

Experiment 7 was a small experiment within experiment 8. In each treatment, leaf laminae were removed at full anthesis from six plants in each of the three plots of Sirosun Bianca Grande. There were five treatments: (1) all laminae; (2) alternate laminae; the (3) upper or (4) mid third of laminae were removed; or (5) all laminae were left intact. Seeds were harvested at physiological maturity (105 DAS), 35 days after defoliation.

Gas chromatography technique for fatty acid analysis

After collection at physiological maturity, seeds from all experiments were dried to constant weight at 70°C. Duplicate fatty acid determinations were made on seeds from each plot, head or sample. About 20–25 g of cleaned seeds from each sample were ground in a Krups coffee mill. A subsample of 200 mg of ground material was placed in a mortar with 1 g of washed and ignited sand and 3 ml of petroleum spirit and ground until pulverised. The supernate was filtered through a non-absorbent cotton and placed in a 10-ml Erlenmeyer flask. The petroleum spirit was allowed to evaporate.

Fatty acid methyl esters were prepared by transesterification with sodium methoxide (Christie 1973). Three millilitres of (0.5 M) sodium methoxide in methanol was refluxed for 7 min with the oil left after evaporating the petroleum spirit. Then the mixture was cooled rapidly and 7 drops of concentrated acetic acid were added to stop the reaction. After 10 min, 5 ml of hexane were added to the flasks and they were left undisturbed for 5 min. Next, water was added to the flasks
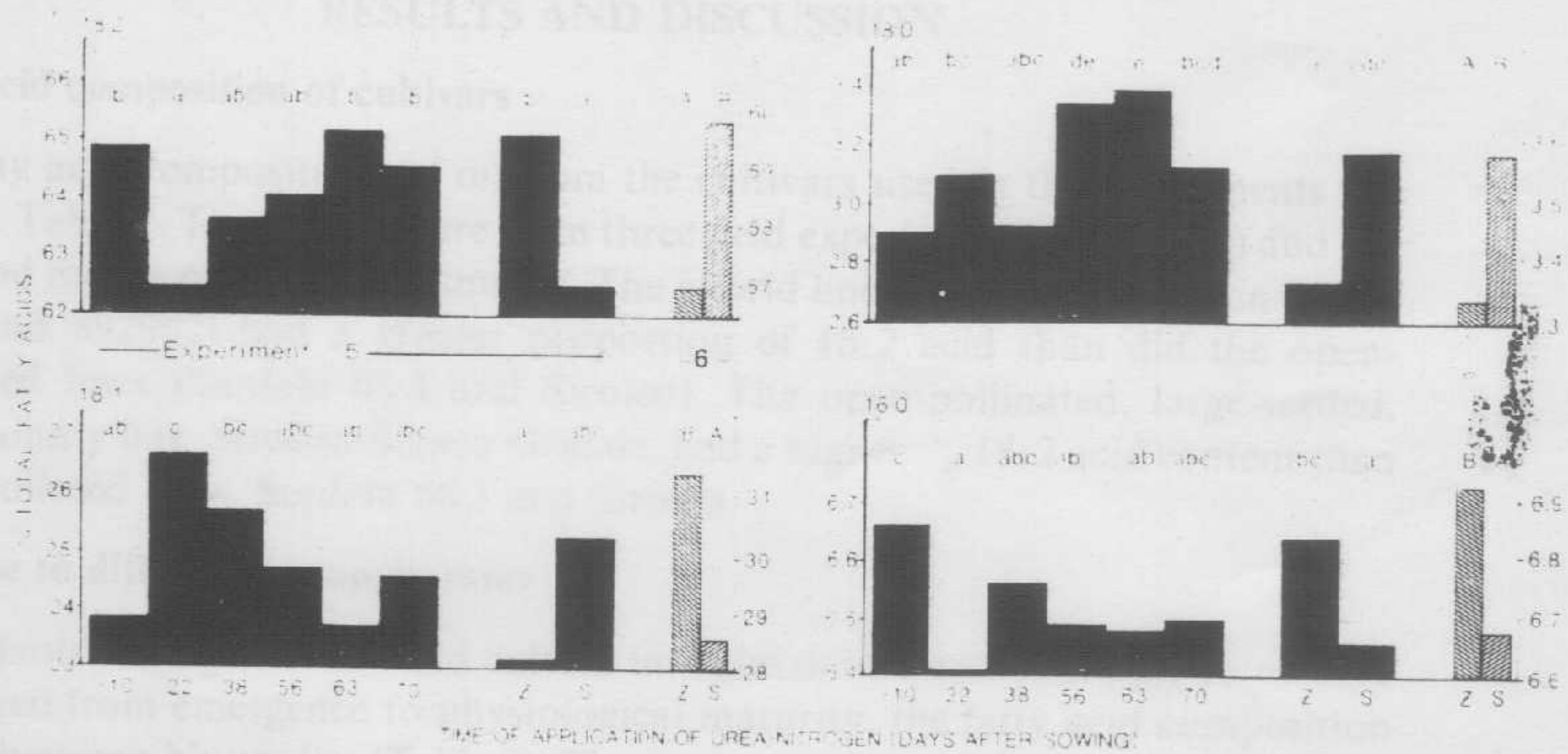
Fig 1. Response of fatty acid composition of seeds to time of N application (Steer and Hocking 1985). In experiment 5 (solid bars, scales on left-hand axes) 120 kg N ha⁻¹ was applied before sowing (10 days after sowing, DAS), or at floral stage 1 (22 DAS), floral stage 8 (38 DAS), early anthesis (56 DAS), full anthesis (63 DAS) or 7 days after full anthesis (70 DAS). S was a split N application in which 20 kg N ha⁻¹ was applied at each of the above times. In experiment 6 (cross-hatched bars, scales on right-hand axes) 50 or 100 kg N ha⁻¹ was applied at floral stage 1 (29 DAS) and at early anthesis (65 DAS). Z was the zero N control in both experiments. Data in experiment 5 are means of Hysun 30 and Sirosun 132H, and in experiment 6 means of Sunfola 68·3, Sirosun 892952 and Sirosun Bianca Grande. Within an experiment values with the same letter above do not differ at $P = 0.05$: lower case for experiment 5 and upper case for experiment 6.

F values from analyses of variance

Factor (degrees of freedom)	Fatty acid			
	16:0	18:0	18:1	18:2
Experiment 5				
Nitrogen (7,28)	2·79*	7·79***	2·51*	3·43**
Variety (1,32)	3·57***	15·66***	11·5**	4·33*
N × V (7,32)	1·85	3·49**	0·69	0·76
Experiment 6				
Nitrogen (1,42)	8·79**	5·68*	8·97*	9·44**

to bring the hexane layer up into the neck of the flask. Flasks were tapped to displace air bubbles. Then, after 5 min, 2 μl of the hexane layer were used for the analysis of the fatty acid methyl esters.

They were analysed on a Packard 421 gas chromatograph fitted with a flame ionisation detector. The column was a 1·8 m × 2·0 mm glass column packed with 10% SP-2320 on 100/120 Chromosorb W AW (Supelco Inc, Bellefonte, PA). Both the injector ports and detector ports were set at 250°C, and the oven operated at 200°C with a N flow of 20 ml min⁻¹. An electronic digital integrator was used to calculate the total area under peaks, and the area of each fatty acid peak was expressed as a percentage of the total area. Fatty acid methyl ester standards (Supelco Inc, Bellefonte, PA) were used to check peak retention times of the various fatty acids.

RESULTS AND DISCUSSION

Fatty acid composition of cultivars

The fatty acid compositions of oil from the cultivars used in the experiments are given in Table 2. These results are from three field experiments (5, 6 and 8) and are the grand means of several treatments. The hybrid lines (Hysun 30, Sirosun 152H, 132H and 892952) had a greater proportion of 18:2 acid than did the open-pollinated lines (Sunfola 68·3 and Siroleo). The open-pollinated, large-seeded, confectionary line, Sirosun Bianca Grande, had a higher % 18:2 acid content than did the oilseed lines, Sunfola 68·3 and Siroleo.

Response to different N supply rates

When plants were grown in sand culture in a glasshouse and N supply rates were unchanged from emergence to physiological maturity, the fatty acid composition differed between N supplies (Table 3). The proportions of 18:2 and 16:0 acids were low on the lowest N supply rates, and 18:1 acid was significantly higher. On 8·7 mg N d^{-1} per plant 18:0 acid was higher than on higher and lower supplies. These data confirm the report of Djemal *et al* (1985) on the responses of 18:1 and 18:2 acids, and extend present knowledge by showing that 16:0 and 18:0 acids also respond to N supply rates. There was a similar response by Sirosun 892952 in experiment 2 (not shown).

Changing N supply rates at certain developmental stages

Two glasshouse experiments were carried out in which the daily rates of N supply were changed at the end of FI (ie at about floral stage 8 (Marc and Palmer 1981), between 35 and 45 DAS). The proportion of the four major fatty acids responded significantly to N supply rates before FI (Table 4). 18:1, 18:2 and 16:0 acids responded to N rates before FI in the same way as they responded to N rates from emergence to maturity (Table 3): lower proportions of 18:1 and higher proportions of 18:2 and 16:0 at high N supplies. The cultivar Sirosun 892952 in experiment 2 (data not shown) responded in a similar manner to Hysun 30 in experiment 1.

By contrast with the hybrid lines in experiments 1 (Table 4) and 2, Sunfola 68·3 in experiment 3 did not show a significant response to N supply rates before FI but it did respond to high rates after FI with a decrease in 16:0 and 18:2, and increase in 18:1 acid (Table 5).

There was a negative response of 18:0 acid to high N supply rates before FI (Table 4) and this was the only acid to show a significant response to N supply rates after FI in three cultivars (Table 6).

These differences between cultivars may well illustrate a fundamental difference in their response to the time of N application because, in field experiment 6, Sunfola 68·3 was the only cultivar to show significantly different responses to N applied at either floral stage 1 or just before anthesis: for % 18:1 (41·2 and 36·1 %, respectively) and for % 18:2 acid (48·1 and 52·5 %, respectively).

Experiment 4 tested the response to changes of N supply rates made at three-row anthesis. 18:1 and 18:2 acids were the only ones to respond significantly to N rates after anthesis (Table 7). This experiment also tested the effect of changing N rates at

TABLE 2

The fatty acid composition of seed oil from seven cultivars grown in three field experiments (values are percent of total fatty acid extracted from the seeds)

	Palmitic 16:0	Stearic 18:0	Behenic 22:0	Lignoceric 24:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
Experiment 5							
Sirosun 132H	6·2[a]	3·9[b]	0·78[b]	0·27[b]	23·9[a]	64·4[b]	0·12[b]
Hysun 30	6·9[b]	2·3[a]	0·68[a]	0·18[a]	25·5[b]	63·7[a]	0·082[a]
Experiment 8							
Hysun 30	6·1[c]	3·3[b]	0·74[a]	0·14[a]	29·6[b]	59·7[bc]	0·097[a]
Sirosun 152H	5·5[b]	4·6[c]	0·87[b]	0·15[a]	25·6[a]	62·8[c]	0·11[b]
Siroleo	5·4[b]	5·4[d]	0·99[c]	0·15[a]	39·5[d]	47·7[a]	0·13[b]
Sirosun Bianca Grande	4·7[a]	2·9[a]	0·86[b]	0·14[a]	33·7[c]	57·0[b]	0·18[c]
Experiment 6							
Sirosun Bianca Grande	7·5[c]	3·8[b]	0·78[a]	0·093[a]	21·2[a]	66·2[c]	0·11[a]
Sirosun 892952	7·0[b]	3·8[b]	0·77[a]	0·13[b]	25·7[b]	62·3[b]	0·11[a]
Sunfola 68·3	5·7[a]	3·1[a]	0·78[a]	0·13[b]	39·3[c]	49·9[a]	0·15[b]

Within a fatty acid and within an experiment, values with the same appended letter do not differ at $P = 0.05$.

TABLE 3

Fatty acid % total extracted from sunflower seeds cv Hysun 30 in response to N supply rates. Experiment 1. (Steer *et al* 1984)

mg NO_3-N d^{-1} per plant	Fatty acid			
	16:0	18:0	18:1	18:2
1·7	5·8[a]	4·9[a]	49·2[b]	39·4[a]
8·7	6·9[c]	5·5[b]	45·8[a]	42·0[b]
21	6·7[c]	4·9[a]	46·1[a]	42·2[b]
42	6·2[b]	5·0[a]	46·5[a]	42·3[b]

Grown in sand culture in a glasshouse. N supply rates were unchanged from emergence to maturity.

[a]-[c] Within a fatty acid, values with the same appended letter do not differ at $P = 0.05$.

See Table 4 for summary of regression analyses.

TABLE 4

The effect of N supply rates before floret initiation on seed fatty acid composition in glasshouse experiment 1 (Hysun 30) (Steer *et al* 1984)

mg NO_3-N d^{-1} per plant	Fatty acid			
	16:0	18:0	18:1	18:2
1·7	6·0[a]	4·8[c]	49·1[b]	39·2[a]
8·7	6·8[b]	4·2[b]	46·4[a]	41·7[b]
21	6·7[b]	3·9[a]	45·6[a]	42·9[b]
42	6·3[a]	3·9[a]	46·7[a]	42·3[b]

Factor (degrees of freedom)	F values			
N before floret initiation (3,63)	7·05***	28·2**	4·91*	7·09***
N after floret initiation (3,60)	1·98	6·14**	1·12	1·19
N before × N after (6,54)	1·74	4·55**	1·63	2·25

Within a fatty acid, values with the same appended letter do not differ at $P = 0.05$.

both FI and anthesis on the same plant (eg low–high–low rates in three developmental phases). These results confirmed that the highest 18:1 proportion (46·0%) was obtained with a combination of a low N supply before FI (as in Table 4) and a high rate after anthesis (as in Table 7). and that 18:2 (maximum = 47·4%) responded in exactly the opposite way to 18:1 acid.

Timing of N application in the field

A field experiment (number 5). using two hybrids, tested the response to single applications of N given at different stages of crop development. There were significant differences between the two cultivars (see Table 2) but except for 18:0

TABLE 5
The effect of N supply rates after floret initiation on the fatty acid composition of seed oil from Sunfola 68·3. Experiment 3

mg NO_3-N d^{-1} per plant	% Total fatty acid		
	16:0	18:1	18:2
1·7	7·3[b]	31·5[a]	58·1[b]
8·7	7·0[a]	36·1[b]	53·2[a]
42	6·8[a]	35·1[b]	54·2[a]

Factor (degrees of freedom)	F values		
N before floret initiation (2.38)	0·54	0·41	0·72
N after floret initiation (2.38)	4·89*	4·67*	5·03*
N before × N after (4.38)	0·28	0·58	0·63

Within a fatty acid, values with the same appended letter do not differ at $P = 0·05$.

TABLE 6
The effect of N supply rates after floret initiation on the 18:0 acid content (% total fatty acids) of seed oil. Experiments 1, 2 and 3, respectively (Steer *et al* 1984)

Cultivar	mg NO_3-N d^{-1} per plant after floret initiation			
	1·7	8·7	21	42
Hysun 30	4·7[a]	4·7[a]	5·0[b]	5·0[b]
Sirosun 892952	—	4·7[a]	4·5[a]	5·0[b]
Sunfola 68·3	1·7[a]	2·1[b]	—	2·3[b]

Within a cultivar, values with the same appended letter do not differ at $P = 0·05$.

TABLE 7
The response of fatty acid composition (% total fatty acids) in Hysun 30 to N supply rates after 3-row anthesis. Experiment 4 (Steer *et al* 1984)

mg NO_3-N d^{-1} per plant after plant anthesis	Fatty acid	
	18:1	18:2
8·7	42·7	45·1
42	44·6	43·1

acid there were no significant interactions between cultivar and N application so means of the cultivars are given in Fig 1. The most noticeable effects were that 18:1 and 18:2 acids responded to the N applications in an opposing manner, and that % 18:2 was highest when no N was applied or when it was applied before sowing or at

anthesis. The lowest % 18:2 acid was obtained when N was applied just before FI (22 DAS), the time at which the maximum response to N of plant mass, seed number and oil yield was obtained (Steer and Hocking 1985). A low 18:2 content was also obtained when the N application was split equally between the six application times.

A comparison between zero N application and applications split between before FI and at anthesis was made in another field experiment, number 6, using cultivars different from those in experiment 5. The responses of % 18:2 and 18:1 acids were opposite to those in experiment 5. The different responses between experiments 5 and 6 cannot be explained unless it is due to cultivar differences.

The proportion of 16:0 acid was depressed by N applications during the growth of the crop. 18:0 Acid responded positively to N applied early in anthesis, consistent with its response to N supply rates between FI and three-row anthesis in glasshouse experiments (Table 6).

Relationships of fatty acid composition with other plant characters

The response of fatty acid composition to the time of N application, especially to N supply rates before FI, raises the question as to whether N is having its effect through other plant characters. For example, N supply rates before FI positively affect leaf and floret numbers per plant, and N rates between FI and anthesis affect single seed weights (Steer and Hocking 1983, 1984; Steer *et al* 1988; Palmer and Steer 1985).

Leaf mass influences the source of C for seed lipid synthesis. Seed number and size would influence the inter-seed competition (Steer *et al* 1988) for precursors of lipid synthesis. Thus, leaf mass, seed number and size could be intermediaries in the effect of N supply, especially before FI, on fatty acid composition. For the four major fatty acids and for these three plant characteristics within the seven experiments (ie for a total of $4 \times 3 \times 7 = 84$ cases) there were only five significant regressions of % fatty acids with either leaf weight at anthesis, seed number or single seed weight; the five were with single seed weight. In addition there were no correlations of fatty acid composition with N or oil concentrations in seeds. It is considered that the few significant correlations were not sufficient to support the hypothesis that N supply rates were acting through other plant characters. The lack of significant differences between different degrees of defoliation is also consistent with this lack of correlation between leaf mass and fatty acid composition.

Defoliation will remove N and C reserves and N metabolic functions in the leaves and limit the supply of new photosynthate for developing seeds. In experiment 7, seeds were collected at physiological maturity from a number of plants of Sirosun Bianca Grande which had been defoliated at anthesis in the field plots of experiment 8. Defoliation was either complete or partial. The latter had either alternate laminae removed all the way up the stem, or all laminae removed from the top or middle third of the nodes. Thus, compared with intact controls, there were varying amounts of leaf laminae remaining at maturity. The concentrations of the four major fatty acids are plotted against the dry weight of leaves remaining at maturity (Fig 2). In no cases were there significant differences between the three partial defoliations, but, over the whole range of leaf dry weight, defoliation caused an
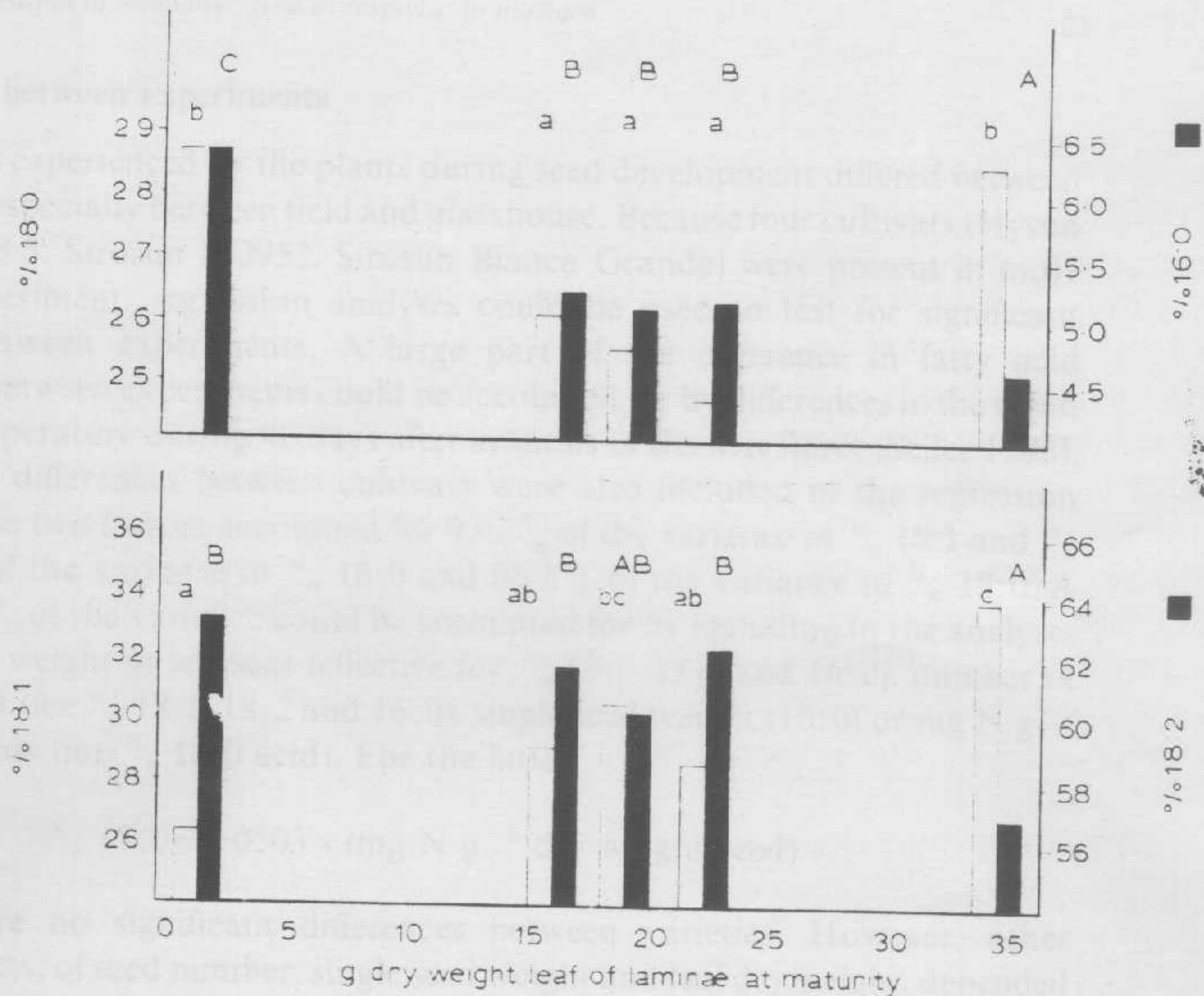
Fig 2. Response of fatty acid composition of seeds of Sirosun Bianca Grande to defoliation at anthesis (Steer *et al* 1988). Experiment 7: the four major fatty acids are plotted against the dry weight of leaf laminae present at physiological maturity. Within an acid, values with the same letter above do not differ at $P = 0.05$; lower case for 18:0 and 18:1 and upper case for 16:0 and 18:2.

F values from analyses of variance

Factor (degrees of freedom)	Fatty acid			
	16:0	*18:0*	*18:1*	*18:2*
Defoliation (4,20)	29.0***	9.86***	4.73**	3.78*

increase in % 16:0 and 18:2 acids and a decrease in % 18:1 acid. Percentage 18:0 acid was significantly higher in intact and completely defoliated plants than in the partially defoliated plants. The changes in 18:1 and 18:2 acids with degree of defoliation are the same as their responses to a low compared with a high N supply rate after anthesis (Table 7), suggesting that defoliation may function not simply by limiting C supply to the developing seeds but also by changing the plant's N metabolism and N supply to the seeds from the leaves.

Seed number per plant and single seed weight were reduced progressively with increasing degrees of defoliation (Steer *et al* 1988) with the result that fatty acid composition was correlated with both of these yield components: % 18:2 and % 16:0 negatively, and % 18:1 acid positively. The weights of fatty acids per seed showed the same trends as % composition.

Relationships between experiments

Temperatures experienced by the plants during seed development differed between experiments, especially between field and glasshouse. Because four cultivars (Hysun 30, Sunfola 68·3, Sirosun 892952, Sirosun Bianca Grande) were present in more than one experiment, regression analyses could be used to test for significant differences between experiments. A large part of the difference in fatty acid composition between experiments could be accounted for by differences in the mean minimum temperature during 40 days after anthesis of the first floret (Seiler 1986), provided that differences between cultivars were also included in the regression analyses. These two factors accounted for 93·2% of the variance in % 18:1 and % 18:2, 87·1% of the variance in % 16:0 and 86·2% of the variance in % 18:0. A further 2 to 5% of the variance could be accounted for by including in the analyses either leaf dry weight at anthesis (effective for % 18:1, 18:2 and 16:0), number of seeds per plant (for % 18:1, 18:2 and 16:0), single seed weight (16:0) or mg N g^{-1} dry weight seeds (for % 18:0 acid). For the latter

$$\% \ 18:0 = 0.0503 \times (\text{mg N g}^{-1} \text{ dry weight seed})$$

but there were no significant differences between varieties. However, other significant effects, of seed number, single seed weight and leaf dry weight, depended upon significant interactions between varieties and the character in question. For example, the effects of seed number and single seed weight depended upon significant differences between Sirosun Bianca Grande and the other varieties, and the effect of leaf dry weight on 18:1 and 18:2 depended on differences between Sirosun 892952 and the other varieties.

The effect of these factors in accounting for the variance in fatty acid composition was small when compared with night temperature and they probably served as within-plant measurements of different N supply rates and timing. The latter could not be used directly in the regression analyses because of the different methods of N application in field and glasshouse experiments.

Practical considerations

The changes in fatty acid composition due to changes in N supply are small compared with the differences due to temperature. Thus with the range of N supplies used in these experiments, 18:1 and 18:2 acids change over a range of 8%, whereas between mean minimum temperatures of 7 and 21°C they change over an 18% range (Harris *et al* 1978). Nevertheless, the changes due to N supply rates could be of commercial significance in some crops. In addition, the interaction of N supply and minimum temperature on fatty acid composition has yet to be investigated.

In order to obtain seeds with a high % 18:2 acid composition, an adequate N supply is needed (Table 3) and it should be provided before floret initiation (Table 4) and perhaps during floret development (Fig 1), but not after anthesis (Table 7). Defoliation at anthesis also produces high % 18:2 acid, but seed and oil yields are depressed severely (Steer *et al* 1988).

The mechanism of fatty acid response to N supply rates

Until the biochemistry of fatty acid synthesis in seeds is better understood (Stumpf 1980) it will be difficult to explain the means by which N supply rates affect the process. A response to the N supply rate during seed development may occur through direct effects of N supply on the enzymes of lipid synthesis. More difficult to explain is how N supply rates before floret initiation affect fatty acid synthesis in seeds that do not develop until some 20–30 days after the completion of floret initiation. Some tissues in the seed are established during floret initiation but then form the seed pericarp at maturity (Vaughan 1970). Although this contains oil it accounts for only a small portion of the oil in the mature seed after the embryo has grown (Harris *et al* 1978). Carbon is probably supplied to the circum-ovule liquid and to the ovule as sucrose (Wolswinkel 1987), so fatty acids are synthesised in the fertilised ovule and not translocated from other organs such as leaves where the early N supply could have influenced synthetic processes. In any case there was no correlation of leaf mass with fatty acid composition of seed oil, except in the defoliation experiment when large portions of the leaves were removed.

A report on fatty acid synthesis in sunflower and rape seeds (Tremolieres *et al* 1982) suggested that there is a genetically programmed activation or synthesis of oleoyl-CoA desaturase, and that environmental factors could interact with this programmed activation. In such a manner early N supply rates could modify the genetic programme that later is expressed as different desaturase activities in the developing seed, but this still leaves unanswered the question of how N supply rates modify the genetic programme for desaturases.

The flux of carbon in fatty acid synthesis occurs from 16:0 to 18:0 to 18:1 to 18:2 to 18:3 acids, where they are present as complexes with acyl carrier protein (ACP), coenzyme A or phosphatidylcholine. Stumpf (1980) has suggested that different proportions of fatty acids in lipids can be interpreted as the differential hydrolysis of these complexes. For example an enhanced hydrolysis of stearoyl-ACP, removing it from the synthetic sequence, would give a relatively high 18:0 content and a low 18:1, 18:2 and 18:3 content. In this scheme N supply would affect the activity of the hydrolytic enzymes.

Stumpf (1980) also reviewed the evidence for the site of synthesis of fatty acids in cells of leaves or seeds and proposed a scheme whereby the steps up to 18:1 acid take place in proplastids and the desaturation of 18:1 to 18:2 and 18:3 acids occurs in the cytosol, associated with the endoplasmic reticulum. Thus the proportions of fatty acids may be controlled not only by the activity of enzymes but also by a transport step from one organelle to another. The N supply rates could affect this transport step. The relationship between the proplastid and cytosol components may be expressed simply by the 18:1/18:2 ratio or more completely in sunflower by the fraction (16:0 + 18:0 + 22:0 + 24:0 + 18:1)/(18:2 + 18:3). The latter is given in Table 8. A low value means a relatively high flux of fatty acids into the cytosolic compartment. It is seen that a high N supply before FI to Sirosun 892952 and Hysun 30 gives the lowest value, suggesting an enhancement at this transport step. Once again, Sunfola 68.3 has responded differently from the hybrid cultivars by showing a significant effect only after floret initiation and by giving the lowest ratio from the lowest N supply.

TABLE 8

The ratios (saturated acids + 18:1)/(18:2 + 18:3) in response to N supply rates. The ratios are ranked in decreasing order and represent acids synthesised in the plastids/those synthesised in the cytosol (according to Stumpf 1980)

Sirosun 892952 Experiment 2[1]		Hysun 30 Experiment 1[2]		Sunfola 68·3 Experiment 3[3]	
N supply before/ after floret initiation	Ratio	N supply before floret initiation	Ratio	N supply after floret initiation	Ratio
8·7/21	1·85[e4]	1·2	1·57[b]	8·7	0·886[b]
8·7/8·7	1·73[de]	8·7	1·41[a]	42	0·846[b]
21/8·7	1·51[cd]	42	1·36[a]	1·7	0·716[a]
21/21	1·45[bc]	21	1·33[a]		
21/42	1·43[bc]				
8·7/42	1·30[abc]				
42/42	1·22[ab]				
42/8·7	1·19[ab]				
42/21	1·12[a]				

[1] A significant interaction between N supply before and after floret initiation.
[2] Only a significant effect of N before floret initiation.
[3] Only a significant effect of N after floret initiation.
[4] Values within an experiment with the same appended letter do not differ at $P = 0.05$.

The detailed mechanism of fatty acid response to N supply must await improvements in our knowledge of lipid synthesis and further experimentation with N supply rates.

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