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LEVELS OF MOISTURE, OIL, NITROGEN AND FATTY ACIDS IN THE MATURING SEED OF SUNFLOWER (HELIANTHUS ANNUUS)

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SUMMARY

Fortnightly measurements of moisture, oil, nitrogen and fatty acids in the maturing seed of sunflower (*Helianthus annuus*) indicated rapid development 0 to 35 days after flowering. Final levels (moisture free) were obtained before seed moisture reached 10%. Lower temperatures during seed maturation favoured increased linoleic acid production.

I. INTRODUCTION

Development of the sunflower seed during maturation has been studied by a number of workers (Hopkins and Chisholm 1961, Robinson 1970). Oil produced from sunflower seed is of particular interest since the levels of the unsaturated fatty acids oleic and linoleic have been found to vary with the temperature during maturation (Kinman and Earle 1964, Canvin 1965, Johnson and Jellum 1972).

As part of a detailed 'time of planting' trial during the 1973-74 season, the maturing seed was analysed for moisture, oil, nitrogen and fatty acid levels.

The trial design required a large number of measurements and observations to be taken by a range of personnel. Because of this, the need arose to restrict sampling of the plant to predetermined fortnightly intervals. These restrictions resulted in some gaps in the data, however the results proved most interesting. Although physical measurements were made during the investigation, this paper only deals with chemical data.

II. MATERIALS AND METHODS

Design—

Five times of planting T_1 = 30 Aug 73
T_2 = 4 Oct 73
T_3 = 13 Nov 73
T_4 = 4 Dec 73
T_5 = 7 Jan 74

Three populations P_1 = 25 000 plants ha^{-1}
P_2 = 50 000 plants ha^{-1}
P_3 = 100 000 plants ha^{-1}

An additional planting T_6 (13 February 1974) was measured at maturity only for chemical analysis.

Samples

For each population, two sample quadrats (Q1 and Q2) were sampled for each time of planting. These sample quadrats were preselected randomly so that each fortnight different quadrats would be sampled, thus reducing the possibility of 'neighbouring effect' resulting from partial destruction of a sampling area.

Approximately 10 whole heads were taken from each sample quadrat. Seeds were removed by hand, taking care to avoid biased sampling. A laboratory winnower was used to clean seeds before moisture determinations were carried out.

Drying

Moisture determinations were carried out at 80°C for 24 hours. Freeze drying was investigated using a DYNAVAC FD 16 freeze drying unit (temperature = −75°C, pressure = 10^{-2} Torr).

Rate of moisture loss was compared with oven drying at 80°C (See figure 1).

Oil content

Rapid grinding of the dry seed in a 'BRAUN' grinder followed by cold hexane extraction of the ground meal proved to be the most satisfactory for immature seed. Details of the method are—

Approximately 30 g of dried seed were ground in a 'BRAUN' grinder for 30 seconds. After mixing, 9 to 10 g were accurately weighed into a stoppered 100 ml measuring cylinder and 100 ml distilled hexane (B.P. 60°C) added. The stoppered cylinder was then shaken (24 h) on an end-over-end shaker.

After removal from the shaker, the cylinder was stood upright to allow solids to settle out. Approximately 80 ml were then decanted into a centrifuge tube (screw cap) and centrifuged at 2 000 r.p.m. for 10 min. Fifty millilitres of the clear hexane were then transferred to a tared 100 ml beaker and allowed to evaporate under a stream of nitrogen. Final drying was accomplished at 30°C in a vacuum oven.

Comparison of samples oven and freeze dried showed no difference in fatty acids even at this early stage of rapid physiological change.

TABLE 1

SUNFLOWER SEED (TWO WEEKS AFTER FLOWERING)

—	% Palmitic Acid	% Stearic Acid	% Oleic Acid	% Linoleic Acid
Freeze Dried	8·7	7·1	53·7	30·6
Oven Dried (80°C) ..	8·6	7·2	53·8	30·5

Soxhlet extraction was used for all samples of mature and near mature seeds.

Distilled hexane was used in preference to diethyl ether, to avoid possible peroxide oxidation of samples.

Nitrogen

Nitrogen determinations were carried out by the Kjeldahl method, using an accurately weighed sample of approximately 2 g.

This sample was taken from the freshly ground, oven-dried seed at the same time as the sample for oil content.

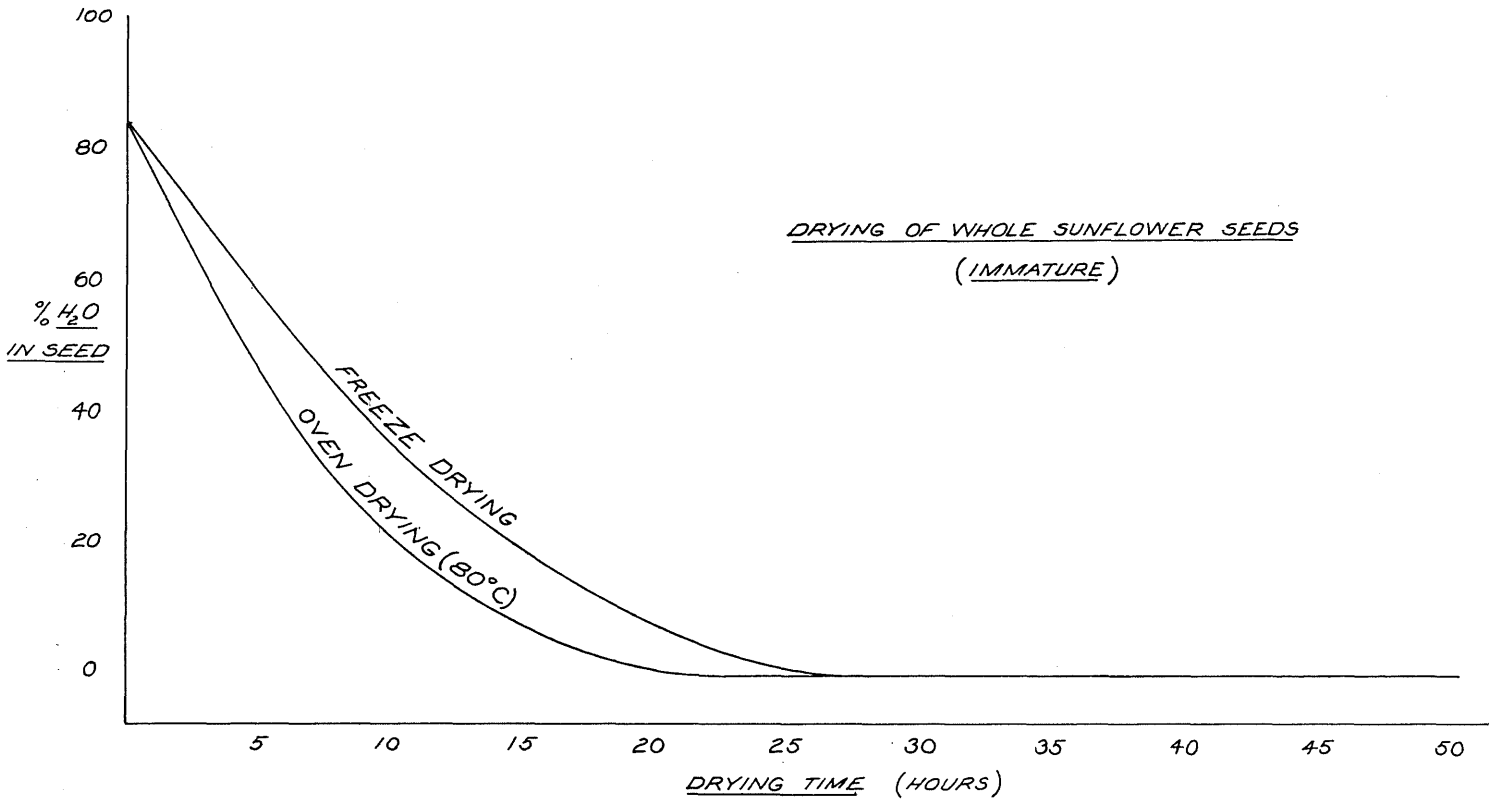

Figure 1.—Comparison of freeze drying and oven drying of whole seed.

Fatty acids

Determined by gas liquid chromatography (GLC) as methyl esters using a PYE, SERIES 104 gas chromatograph.

Methyl esters

Esterification of oil samples (0.1 g) was achieved using boron trifluoride and methyl alcohol (BF_3/MeOH) based on the method of Price (1968).

Methyl ester standards

All standard were purchased >99% pure and each checked for purity by GLC.

GLC operating conditions

Esters were separated on a 1.52 m x 0.64 cm stainless steel column packed with diethyleneglycol succinate (D.E.G.S.) on silanised Embacel. Injector temperature 250°C, Column temperature 195°C, Hydrogen flame ionization detector (FID) temperature 250°C.

Sample application

Sample and standards were injected alternatively (8.5 ± 0.1 μl) using a fixed needle Hamilton syringe. The total weight of the sample injection did not exceed 50 μg and linear response was obtained within this range.

III. RESULTS AND DISCUSSION

In all cases, no significant difference was observed between the three populations studied, irrespective of the stage of growth or time of planting. As a result, the figures quoted in this paper represent the mean of six separate analyses from six separate samples, for example,

$$\text{MEAN FOR } T_1 \text{ (7 days)} = \frac{T_1P_1Q_1 + T_1P_1Q_2 + T_1P_2Q_1 + T_1P_2Q_2 + T_1P_3Q_2 + T_1P_3Q_2}{6}$$

Figure 2 shows the moisture status of the seed from shortly after flowering to complete maturity. In all cases, the moisture level dropped rapidly during the 5 weeks after flowering and then levelled off to approximately 7%. It can be seen that the moisture loss for T_5 was slightly slower than for the others. This was no doubt due to the lower temperatures experienced by T_5 during the first 3 weeks. (See table 2).

Figures 3 and 4 show the accumulation of nitrogen and oil in the seed. These figures represent the actual amount in the seed as it existed and not moisture free. It can be seen that a rapid accumulation occurred during the first 5 weeks, corresponding to the rapid decrease in moisture level. The accumulation of both nitrogen and oil for T_5 is shown to be slower than that of the others. Examination of figure 5 shows that the slow nitrogen accumulation as shown in figure 3 was due to the slower rate of moisture loss (figure 2). The moisture free nitrogen figures for T_5 (figure 5) were significantly higher than the others shown. This high nitrogen figure for T_5 corresponds to the low oil figure for T_5 as illustrated in figure 6.

Examination of figures 5 and 6 shows that after approximately 5 weeks, the nitrogen and oil have reached their final value. This point corresponds to the same stage where moisture level slightly exceeds 10%. Seed weights were not measured; however, published work (Hopkins and Chisholm 1961) shows that seed yield (moisture free) would follow a similar pattern.

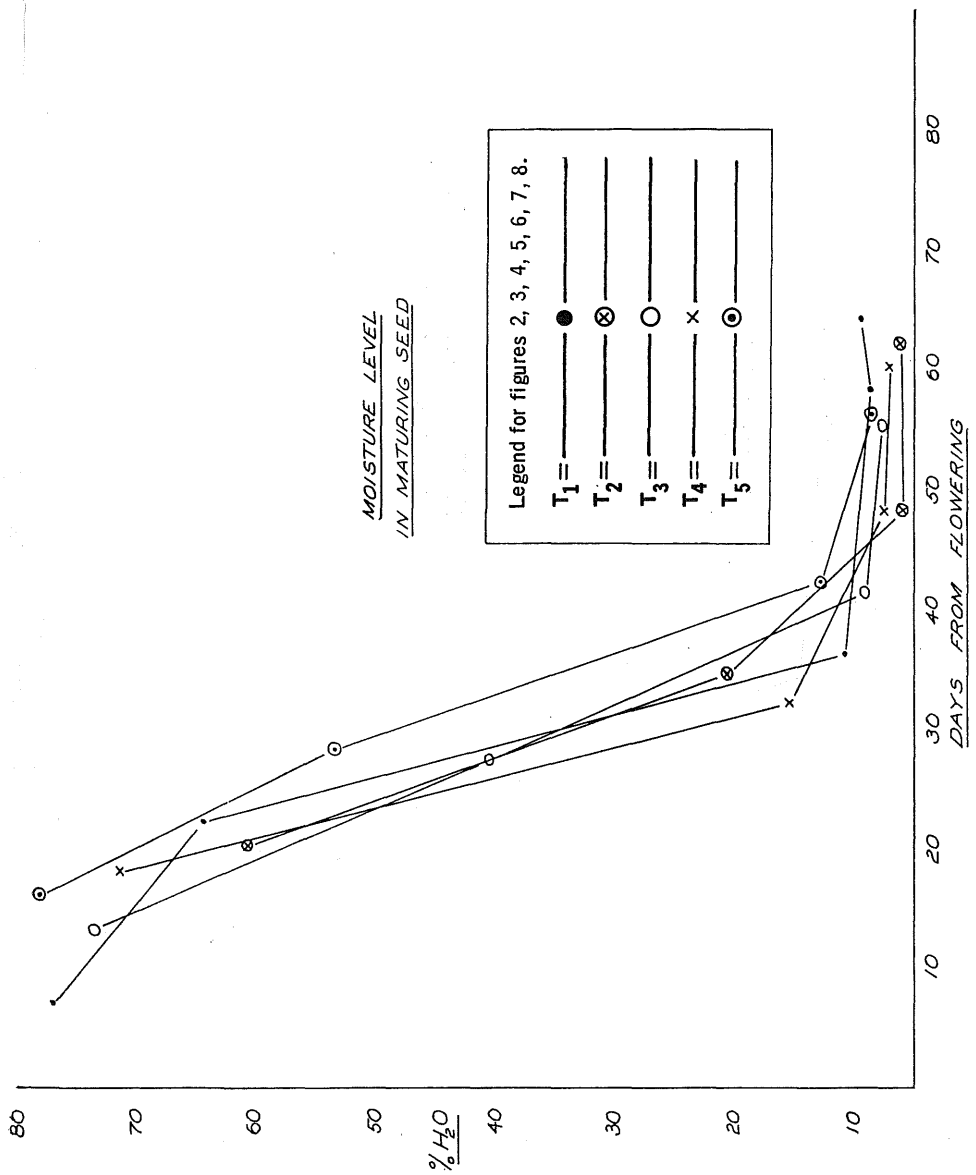

Figure 2.—Moisture level of seed during maturation.

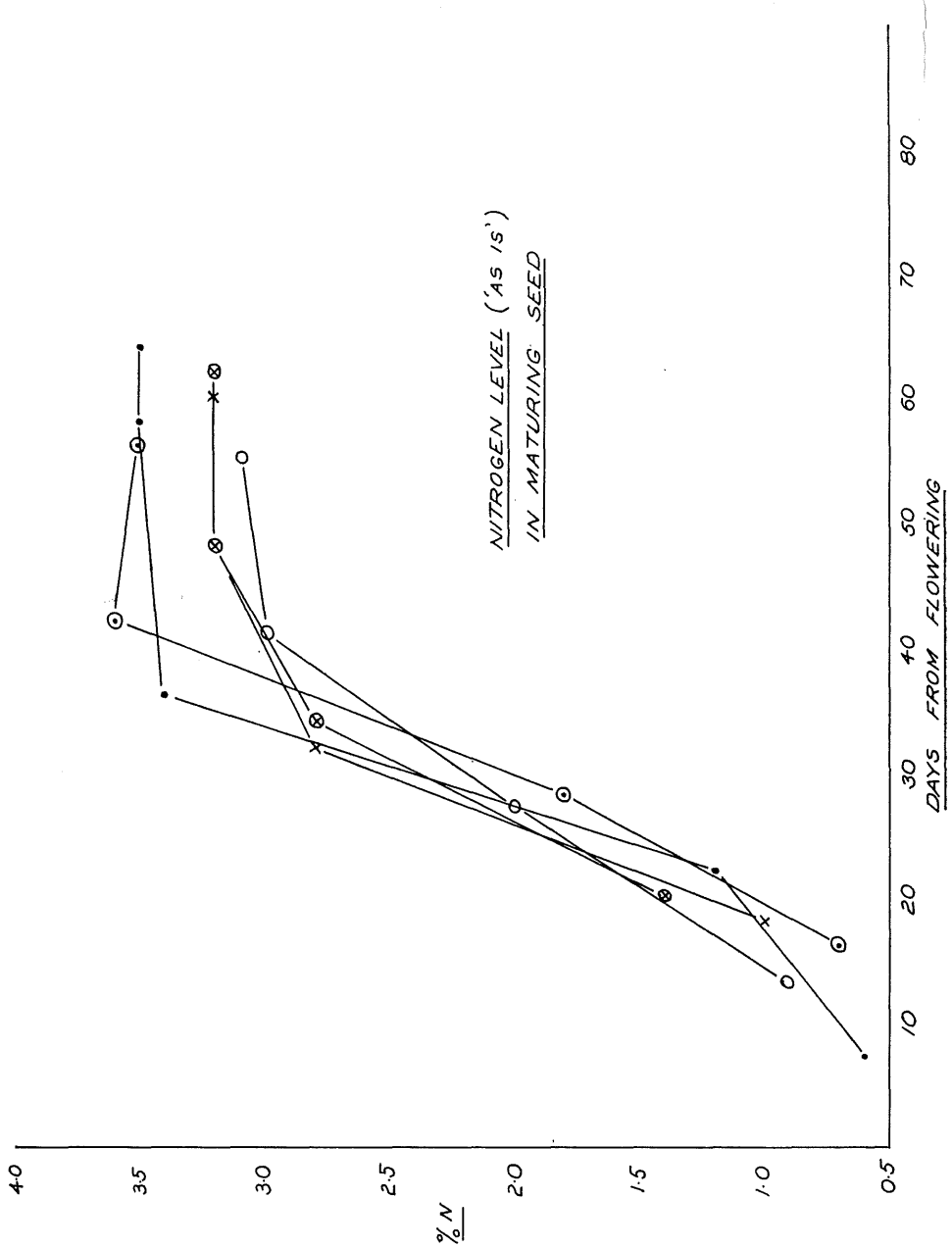

Figure 3.—Nitrogen level 'as is' during maturation.

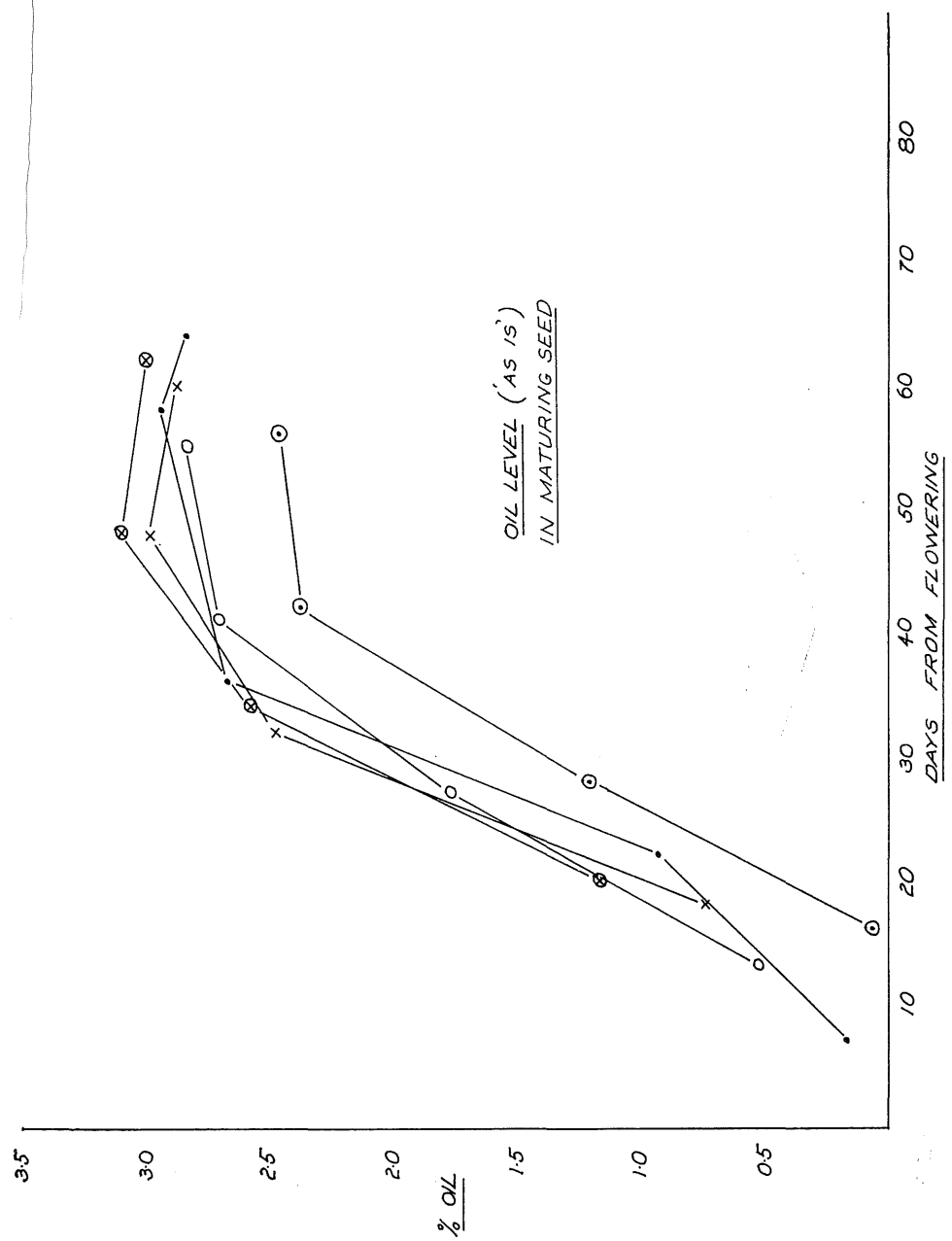

Figure 4.—Oil level 'as is' during maturation.

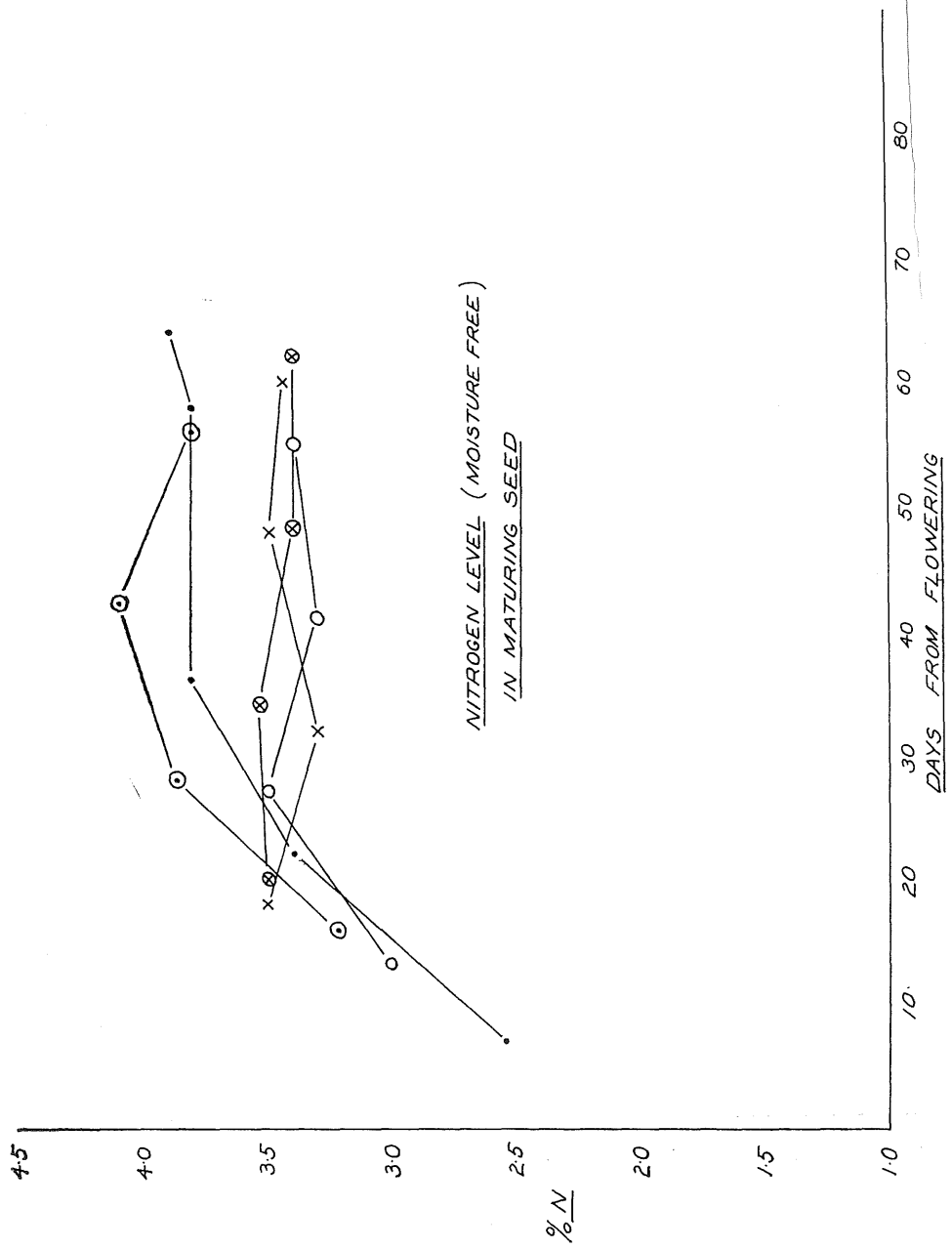

Figure 5.—Nitrogen level (moisture free) during maturation.

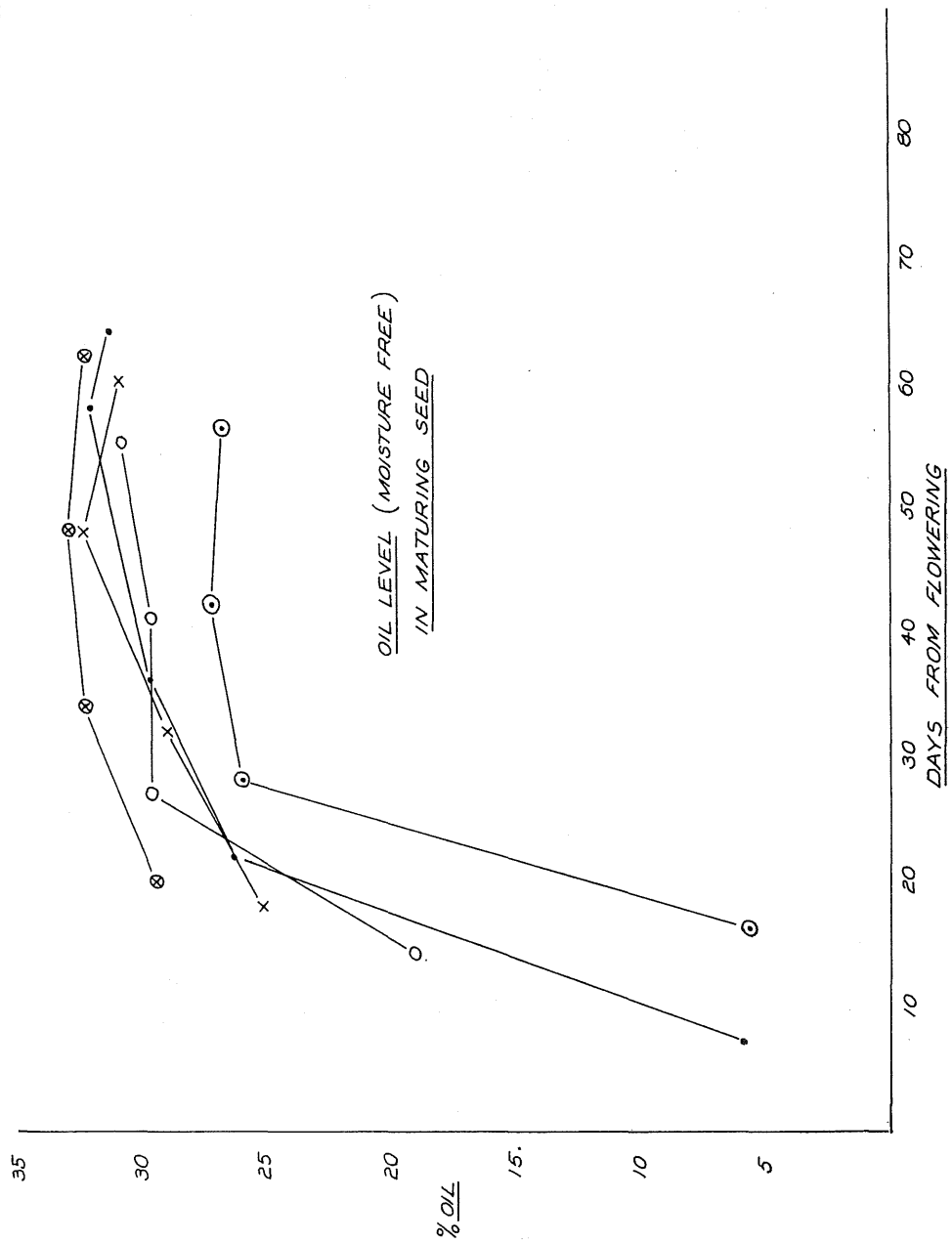

Figure 6.—Oil level (moisture free) during maturation.

These data indicated that once seed moisture has reached 10% the crop could be harvested, provided that the surrounding foliage, including the head itself, was not so moist as to produce mechanical harvesting problems.

Figures 7 and 8 show that, after 35 days, the levels of the two unsaturated fatty acids oleic and linoleic have reached their optimum value. It is important to note here that the levels of the saturated fatty acids palmitic and stearic were measured for all samples, but since no significant changes were observed, these results were not recorded in this paper.

For all times of planting, the level of oleic acid decreased while the level of linoleic acid increased as the seed matured. At very early stages, for example, less than 10 days after flowering, the level of oleic acid was greater than the level of linoleic acid.

It was expected that, over the whole range of planting dates, at least one planting, would experience hot conditions to produce a low final level of linoleic acid. Unfortunately, conditions were mild throughout (table 2) and final levels were similar for all planting times. It would have been of interest to see whether under hot conditions, a slow linoleic build-up occurred or whether in fact a peak was produced which then dropped back to a low level. It is hoped to examine this at a later date.

TABLE 2

TEMPERATURE DATA FOLLOWING FLOWERING

Time of Planting	Date of 50% Flowering	Mean Daily Temperature (°C)		
		1–3 Weeks after 50% Flowering	2–4 Weeks after 50% Flowering	3–5 Weeks after 50% Flowering
30 Aug 73 (T_1)	5 Nov 73	22·2	22·7	22·4
4 Oct 73 (T_2)	4 Dec 73	23·3	24·1	24·6
13 Nov 73 (T_3)	9 Jan 74	22·1	21·8	22·6
4 Dec 73 (T_4)	1 Feb 74	23·8	23·4	21·8
7 Jan 74 (T_5)	5 Mar 74	20·9	21·7	22·6
13 Feb 74 (T_6)	13 Apr 74	17·2	15·7	16·4

Table 3 shows the final level of oleic and linoleic acids for each time of planting. It can be seen that very little change occurred apart from an expected change in T_6 which was the only planting to experience a significantly different temperature during maturation.

TABLE 3

FATTY ACID LEVELS AT MATURITY

Time of Planting	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid
30 Aug 73 (T_1)	6·8	4·4	21·3	67·6
4 Oct 73 (T_2)	6·1	3·6	20·5	69·8
13 Nov 73 (T_3)	5·4	3·7	21·4	69·7
4 Dec 73 (T_4)	7·1	4·3	24·7	63·9
7 Jan 74 (T_5)	7·4	4·6	19·9	68·3
13 Feb 74 (T_6)	6·4	3·8	14·3	75·4

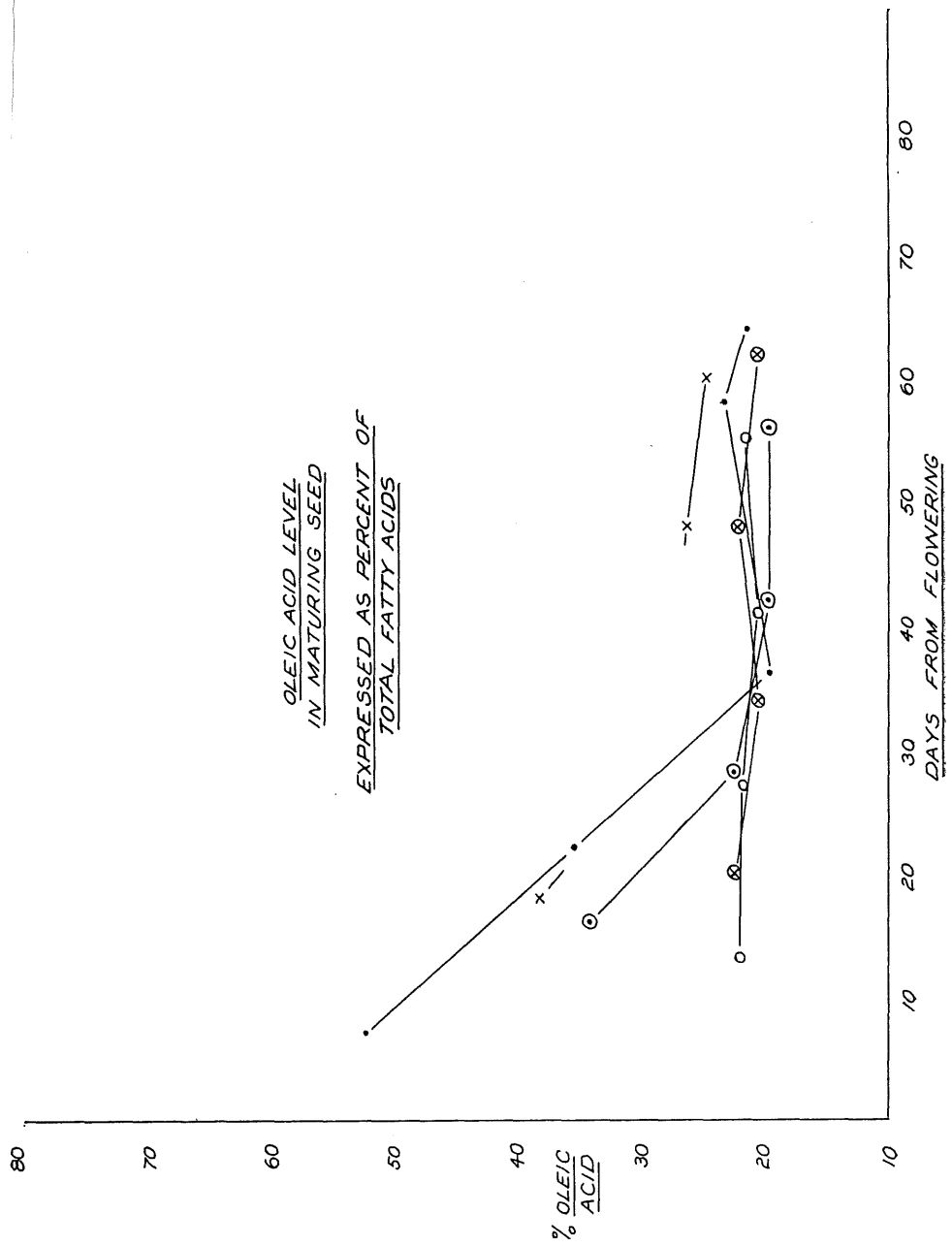

Figure 7.—Oleic acid level in the oil of maturing seed.

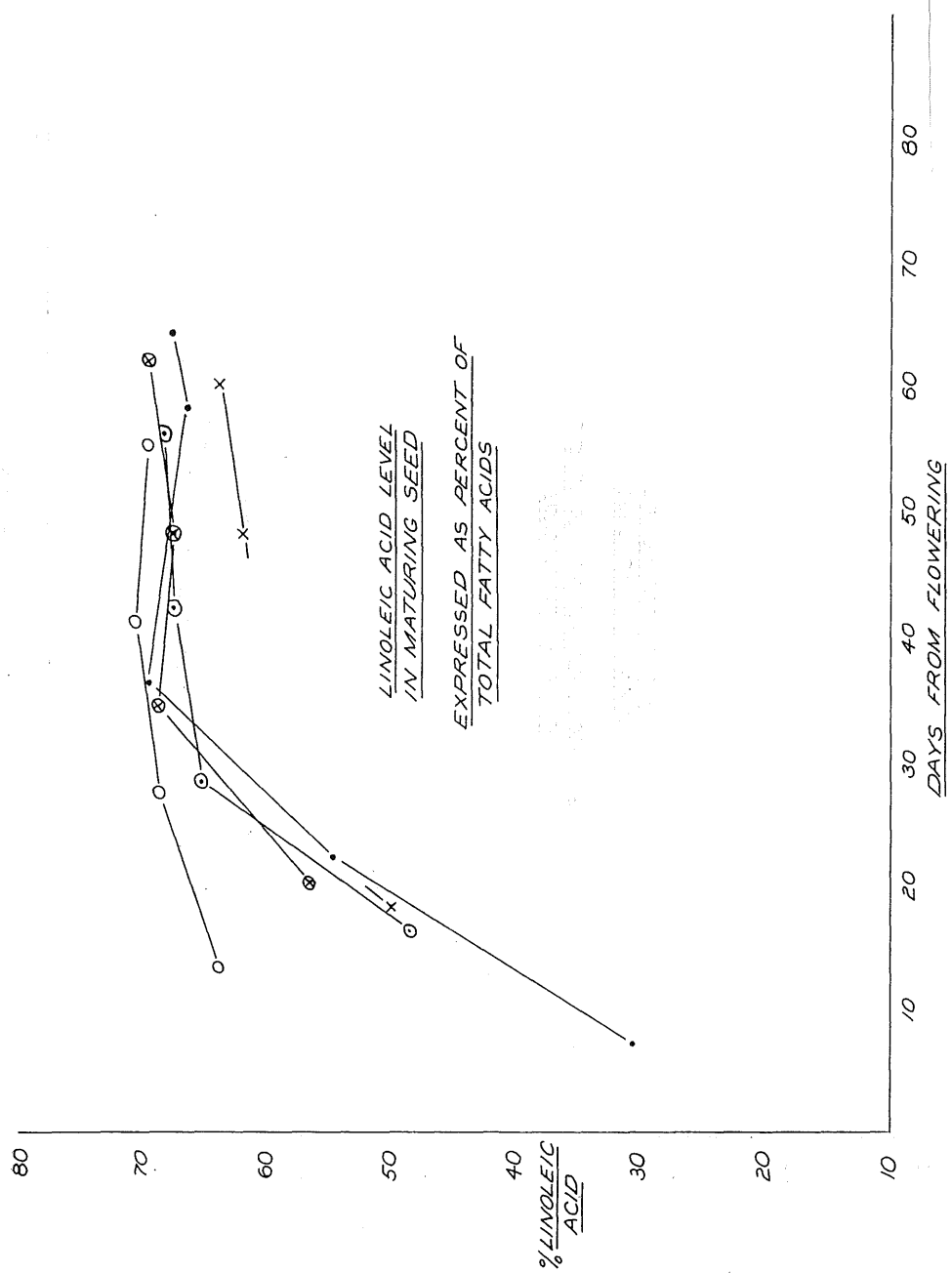

Figure 8.—Linoleic acid level in the oil of maturing seed.

Some explanation must be given for the extremely low levels of oil produced at all phases of this experiment. Because of the nature of the experiment, the variety 'Issanka' was selected primarily because of its uniformity, in both vegetative growth and flowering. This variety has, in Queensland at least, a history for producing low oil levels. In one variety trial conducted at the Biloela Research Station 1972-73, 'Issanka' produced the lowest oil (26.9% M.F.), of 13 varieties tested. 'Sunfola 68-2' produced 40.6% M.F. in the same trial.

Because such low oil figures were obtained in our experiment, it was decided to check the planting seed in both field and glasshouse experiments. Table 4 indicates that, under certain conditions, the planting seed can produce high oil levels. The extremely high levels produced under glasshouse conditions show clearly that this variety is sensitive to some environmental or soil factor which at present remains unexplained. Further work is being carried out in an attempt to establish the important limiting factor.

TABLE 4

OIL CONTENT DATA ON VARIOUS SEED SOURCES

Planting Seed		Toowoomba (Glasshouse)	Emerald (Field)
Seed Source	% Oil (MF)	% Oil (MF)	% Oil (MF)
Issanka (1)	34·0	54·3	38·9
Issanka (2)	33·0	52·9	40·1
Issanka (3)	37·0	51·2	39·7
Issanka (4)	33·0	49·4	41·1
Sunfola 68–2 (5)	42·0	57·5	43·9

Seed Source—1: Planting seed used for main trial.
2: Daughter seed from 1.
3: Similar to 1 but from a different bag.
4: From a seed increase 1971–72.
5: Sunfola 68–2 harvested 1972–73.

Despite the low oil figures obtained in the main experiment, the results provide valuable information on the chemical changes occurring during seed maturation.

IV. ACKNOWLEDGEMENTS

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