SELECTION OF SUNFLOWER PLANTS CONTAINING HIGH LINOLEIC ACID, AND ITS AGRONOMIC SIGNIFICANCE.

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ABSTRACT

Variation in the ability of plants to produce a high percentage of linoleic acid in the oil when grown under a range of temperature conditions was investigated in sunflower.

A relationship between the level of linoleic acid in the cypsela (seed) of the mature plant and rate of germination of its seedling at 4°C was established. This can be used to select for high linoleic acid. Selected plants and families were capable of producing 70 to 80 percent linoleic acid under temperature conditions in which the original population averaged only about 40 percent linoleic acid.

Agronomic and genetic implications of this relatively temperature-stable linoleic acid synthesis pathway are briefly discussed.

INTRODUCTION

Sunflower, *Helianthus annuus* L. is widely used as a source of polyunsaturated vegetable oil. In margarine, for example, polyunsaturation is claimed if the oil contains 40% polyunsaturated fatty acids. (e.g. linoleic) and 20% saturated fatty acids (e.g. palmitic). But to provide a suitable texture at least 62% linoleic acid is required.

However Kinman and Earle (1964) concluded that, within variety, linoleic acid content varied according to the climatic conditions under which the seed was produced. Canvin (1965) demonstrated in one variety, that under constant 10°C conditions sunflower produced almost 80% linoleic acid, but at 26.5°C the linoleic acid level dropped to about 25% and the oleic acid content increased substantially.

In a cool environment where most varieties produced 70% linoleic acid, Putt *et al.*, (1969) found that some lines contained only 47% linoleic acid, and concluded that oil quality might be modified by breeding. There is apparently no report of variation in linoleic acid content in lines grown under hot conditions.

During the critical period between pollination and maturity when temperature affects oil quality (Canvin, 1965), it is difficult to avoid hot conditions in many parts of the world. The average daily temperature may exceed 20°C even for winter sown early-maturing genotypes in a Mediterranean-type climate (Downes, 1974). Mean temperatures of 24 — 27°C are experienced by summer crops in many Australian, Argentinian and southern U.S.A. growing areas. Predictions of linoleic acid content of Australian sunflower oil in relation to environmental conditions are presented by Harris *et al.*, (1980).

It clearly would be an advantage to have a sunflower seed that contains a high percentage of linoleic acid irrespective of the environmental conditions under which the cypsela development takes place. What is needed in sunflower is, in fact, a largely temperature-stable linoleic acid synthesis pathway such as occurs in safflower, *Carthamus tinctorius* L. leading to 75 — 82% linoleic acid content (Knowles, 1972). This pathway if available in sunflower, would provide a good deal more flexibility with respect to sowing time and yet provide oil high in linoleic acid. This is important in the subtropical and warm temperate zones where sunflower crops are grown under variable and unreliable rainfall conditions.

In this paper the search for a temperature-stable, high linoleic acid synthesis pathway will be described.

MATERIALS AND METHODS

Experiment 1

A preliminary phytotron experiment was conducted to observe the effect of temperature during seed development on fatty acid composition and to determine the amount of variability obtainable from an experimental variable open-pollinated germplasm ('Siroleo'). This was developed in the CSIRO Division of Plant Industry from early-flowering segregrates from a large number of accessions. The seed used was produced in the field where it matured under average daily temperature conditions about 24°C.

Seed was sown in 15 cm pots containing a 50/50 mixture of perlite and vermiculite. Plants were raised in a phytotron under 24/19°C day/night temperature conditions and were provided with a modified Hoagland's Nutrient Solution. Photoperiod was 16 hours — consisting of natural photoperiod extended by light from incandescent bulbs which provided 50 f.c. at plant height.

At flowering, plants were randomly cross-pollinated and 8 plants (replications) were randomly allocated to 1 of 4 treatment groups. The treatments were temperature conditions: 33/28, 27/22, 21/16 and 15/10°C during seed development. Seed was harvested at maturity from individual plants and ten seeds were taken at random from each plant to provide a composite sample for the determination of fatty acid composition.

Seeds were crushed and methylated with sulphuric acid — methanol (Welch, 1977). Chromatography of the esters was carried out using a Varian Aerograph 204B gas chromatograph, equipped with a stainless steel column packed with Silar 10C on Gas Chrom Q. Peak areas were obtained using a Varian CDS-111C data system. Standard methyl esters were obtained from Applied Science Laboratories Inc.

Experiment 2

Since linoleic acid is formed at the expense of oleic acid under low temperature conditions, it is of interest to determine whether or not fatty acid composition of seed has any effect on the physiology of germination.

Fifty seeds from each plant raised in experiment 1 were placed on moist filter paper in petri dishes. Seed was germinated in a cold room at 4°C. Germination was judged to have taken place when the radicle had achieved a length of 5 mm., a variation of the procedure of Gimeno (1974). Counts of germinated seed were made daily. An estimate of the days to median germination was determined by summing, over the germination period, the product of number of days to germination and the proportion of viable seeds which germinated on the particular day.

To investigate stability for high linoleic acid content, plants were selected because they produced seed of high linoleic acid content or they had been pollinated by plants with high linoleic acid content in seed. Linoleic acid levels of these plants varied from 39% to 83% but all were expected to give high linoleic acid levels in their progeny.

Seed of each of the selected plants was sown in pots as in experiment 1 and plants were raised to flowering at 24/19°C. Six plants (replications) from each family were allocated randomly to each of three treatment groups (180 pots) which were: temperature regimes after flowering either 27/22, 21/16 or 15/10°C. Plants were pair-crossed so that all male and female parents could be identified. At maturity, seed was taken from each plant individually and 10-seed samples were used to determine fatty acid composition.

RESULTS

Analysis of the fatty acid content of the seeds obtained in experiment 1 revealed the same inverse relationship between oleic and linoleic acid content as reported by Canvin (1965). The results suggested however that linoleic acid did not increase gradually with cooler conditions but rather that it was low (about 42%) under all temperature regimes 15/10°C at which oil of 75% linoleic acid was produced (Table 1).

Although it is generally accepted that linoleic acid is negatively correlated with temperature, the relationship of linoleic acid content and temperature during seed development is extremely variable. Linear correlations have been found in field studies, Harris *et al.*, (1978) for minimum temperatures and Goyne *et al.*, (1979) for mean temperatures; curvilinear relationships in the field for mean temperatures (latter authors) and in controlled conditions (former authors); and no correlations with temperature in the field Johnson and Jellum (1972) and in controlled conditions (Downes, 1974). Canvin (1965) found that between 10° and 21°C, linoleic acid content dropped from 75% to 35% and in raising the temperature to 27°C, a further drop of 10% occurred, a result which is in agreement with the results presented here. Canvin also grew plants with the same day-length as the plants reported here and was of the opinion that the effect of photoperiod on linoleic acid production has been largely neglected.

Table 1. Effect of temperature during seed development on linoleic acid content

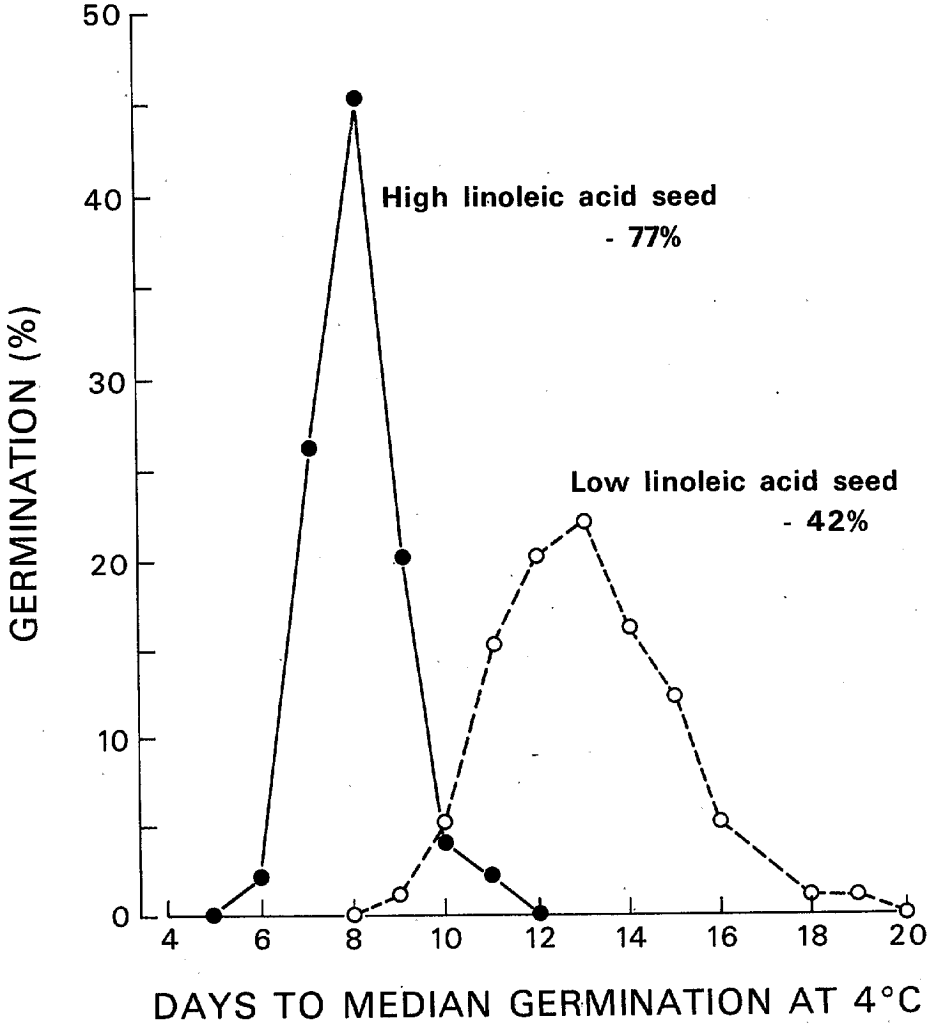
Temperature (°C)	% linoleic acid mean	range
15/10	75.5 ± 4.0	69 — 80
21/16	43.2 ± 5.8	38 — 56
27/22	41.2 ± 14.1	27 — 66
33/28	42.2 ± 7.3	32 — 54

Further, table 1 shows that in the open-pollinated population used there was a good deal of variation in the linoleic acid content of the oil. The greatest range was observed at 27/22°C where, although plants averaged 41%, individuals varied between 27 and 66% linoleic acid. This provided encouraging evidence that linoleic acid content might be readily improved by selection.

When seed produced in experiment 1 was germinated at 4°C, there was a clear relationship between time of median germination and linoleic acid content. Seeds with high linoleic acid content germinated much more rapidly than those with low linoleic acid.

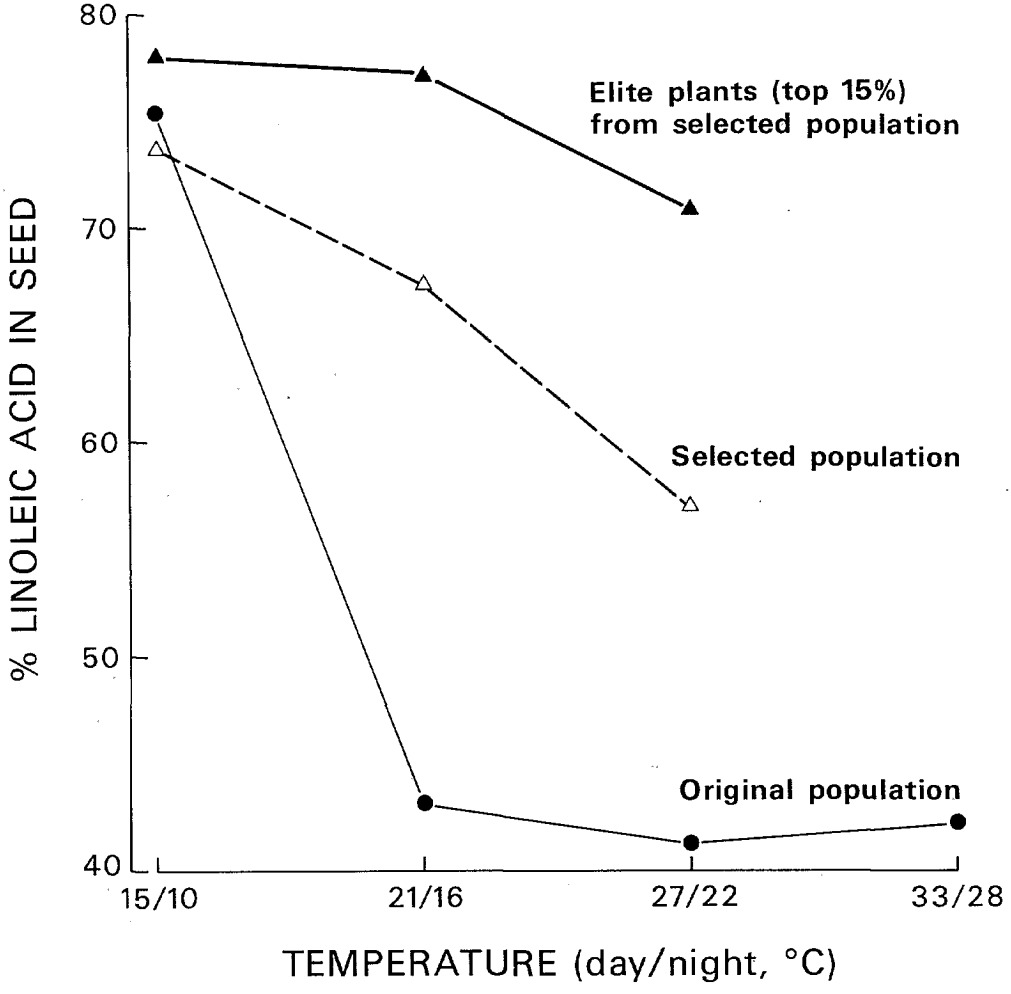
The possibility that time of median germination at 4°C can be used as a selection parameter to identify seedlings with high linoleic acid content is indicated in Figure 1. The time of median germination of seed of six plants containing about 77% linoleic acid is compared with that of six plants containing about 42% linoleic acid. It can be seen that if the fastest germinating seedlings are selected, there is an excellent chance that they contain high linoleic acid. To use this selection technique it is necessary to produce seed under high temperature conditions so that the bulk of the seed can be expected to contain low linoleic acid and will germinate slowly. However the seeds that contain high linoleic acid can be expected to germinate more rapidly. Fast germinating seedlings can be selected and grown on. (Downes and Tonnet, 1982).

Figure 1. Progressive germination at 4°C, of seed of 6 plants containing about 77% linoleic acid (solid line), compared with 6 others containing about 42% (dotted line).



In the last experiment, 10 progenies were grown at three temperature conditions to observe the effect of temperature on the level of and stability of linoleic acid production. Selection has been successful in that the average level of linoleic acid content at 21/16°C and 27/22°C is significantly different from the means of the original population. The average of the ten plants with the highest linoleic acid however, were 78, 77 and 71% at 15/10, 21/16 and 27/22°C respectively suggesting that in these plants, the linoleic acid synthesis pathway appears to be reasonably temperature stable. (Figure 2)

Figure 2. Effect of temperature during seed development on linoleic acid in seed of the original, selected and elite populations.



DISCUSSION

Gas chromatography, iodine number and refractive index provide means of identifying seeds containing different proportions of fatty acids in the oil. However, these methods are slow and are not suitable for screening large populations.

In the search for a temperature-stable linoleic acid synthesis pathway in sunflower neither the frequency nor the action of the gene sought was known. However the association between linoleic acid content and rate of germination under low temperature conditions observed in experiment 2 provides a valuable selection tool.

To account for the increased rate of germination of seed containing high levels of linoleic acid at 4°C, it may be significant that linoleic acid melts at −12°C whereas oleic acid melts at 14°C, and in the seed the contents of the two acids are inversely related. However, seed is likely to contain mixtures of glycerides of oleic and linoleic acid rather than the pure fatty acids, during germination. The seed containing high linoleic acid may germinate more rapidly not because of physical characteristics of the oil but because linoleic acid may be more rapidly mobilised than oleic acid in the germination process.

The population 'Siroleo', used in the search for seeds containing high levels of linoleic acid at high temperature may not be typical of other open-pollinated sunflower varieties. It had been developed by collecting seed from early maturing plants after winter sowings. Thus there may have been selection for rapidly germinating seeds — this behaviour having been conditioned by the high variability of the linoleic acid content in the original population.

Effect of environment on the unsaturation of oil is not restricted to sunflower. Canvin (1965) for example noted that although the oil of safflower and castor was not affected by change of temperature, that of rape and flax, as well as sunflower, was. Canvin claimed that it is generally accepted that when a plant is grown under a cold climate the seed tends to produce a more highly unsaturated fat than when it is grown under a warm climate. Kawanabe (1979) drew attention to the predominance of unsaturated fatty acids in cool climates and saturated acids in crops of warm environments.

It seems possible that as in the case of sunflower, selection in other crops like soybean and cotton will make it possible to modify their fatty-acid make-up. An important practical benefit from the development of highly unsaturated oil lines may be that the more rapid germination under cool conditions allows crops to be sown earlier.

Since some plants generated in these studies (figure 2) contain above 70% linoleic acid at temperatures as high as 27/22°C, it is likely that the appropriate genes can be incorporated into sunflower varieties to make oil quality more predictable and to improve the flexibility of sunflower production in allowing the planting season to be extended without the expectation of an oil quality penalty. The gene action and inheritance of the apparently temperature-stable

linoleic acid synthesis pathway described here is under investigation. The findings together with an assessment as to how this system might best be used in commercial open-pollinated and hybrid cultivars will be presented in a subsequent paper.

ACKNOWLEDGEMENTS

We are grateful to Mr Simon Mills for technical assistance and to the Australian Oilseeds Research Committee for financial support.

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SELECTION IN SUNFLOWERS FOR RESISTANCE TO RUTHERGLEN BUG *(NYSIUS VINITOR).*

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ABSTRACT

Because of the damage done to sunflower crops in Australia by the Rutherglen Bug, resistant varieties are required. Plants in populations of wild roadside sunflowers which have been exposed to the bug for many years tolerate and resist attack. In progenies of crosses between wild selections and cultivated sunflowers, variation for resistance was observed and selections made. From these, elite families were selected on germination percentage and oil content. The resistance genes are being transferred into a suitable background to allow their use in commercial varieties.

INTRODUCTION

The Rutherglen Bug (*Nysius vinitor* Berg.) is a member of a genus of plant-attacking *Hemiptera* with world-wide distribution (Slater, 1964). *N. ericae*, the false chinch bug is a serious pest in semi-arid parts of the United States (Milliken, 1918), while the Wheat Bug (*N. huttoni*) damages the New Zealand wheat crop (Burnett, 1976).

In Australia, the Rutherglen Bug breeds in temporary habitats, particularly on seeds of annual plants (Kehat and Wyndham, 1972) and flies to new areas in response to adverse conditions (Kehat and Wyndham, 1973). Thus as winter-growing plant species mature large populations of bugs migrate to sunflower crops.

It is often a week or two before flowering is expected when the bug is first noticed, clustered on the peduncles of the developing buds. Soon wilting can be observed and the reproductive apex may die. Control is difficult because insecticides used about flowering time can kill pollinating insects and the highly mobile Rutherglen Bug can rapidly recolonize sprayed areas. Thus an attack just before flowering causes substantial losses to the farmer.

After the plant has flowered the bugs live on the capitulum, among the bracts and on the developing seeds. It is thought that seed attack induces an increase in free fatty acids which detract from oil quality. The effects of Rutherglen Bug on oil content and seed viability do not appear to be documented. But despite the lack of knowledge of the bug and its effects, it does constitute a serious pest against which it would be useful to have resistant varieties. Indications that this might be possible were provided by Greaves and Rochford (1946) who observed variation in susceptibility among potato varieties. More particularly, encouragement is provided by the success in breeding for resistance to the related chinch bug, *Blissus leucopterus* Say. in maize (Flint, 1921), in sorghum (Snelling *et al.*, 1937) and in wheat (Jones, 1937). In this paper we will describe efforts to locate and develop sources of resistance to Rutherglen Bug in sunflowers.

METHODS

Although the genus *Helianthus* is not native to Australia, naturalized populations of *H. annuus* are widely distributed. They exhibit the dominant wild-type branching from the lower nodes of the wild species rather than the solitary heads and recessive branching which might be expected in escapes from cultivation. Nevertheless in some areas considerable introgression between wild and cultivated forms can be