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Felicity F. Vear. Effet de la stérilité mâle sur la teneur en huile et le rendement en grains chez le tournesol (*Helianthus annuus L.*). *Agronomie*, 1984, 4 (9), pp.901-904. hal-02724857

HAL Id: hal-02724857

<https://hal.inrae.fr/hal-02724857v1>

Submitted on 2 Jun 2020

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The effect of male sterility on oil content and seed yield in sunflowers (*Helianthus annuus* L.)

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SUMMARY

Measurements of oil content and seed yield of some experimental sunflower hybrids between a tester line and individuals from a population showed that the male sterile hybrids contained a mean of 2.62 % more oil than male fertile hybrids and had a higher seed yield (103.19 % of the control varieties compared with 94.97 %). Similar differences were found between inbred male sterile plants and their isogenic maintainers under self pollination (1.25 % more oil, 4.11 g/plant more seed) and cross pollination (1.13 % more oil, 7.56 g/plant more seed). The greater oil content and seed yield of male sterile plants did not appear to be related to the cytoplasm determining male sterility nor to cross pollination it could be due to the male sterility as such.

Additional key words : Hybrid, inbred line, self pollination, cross pollination, chemical, castration, cytoplasm.

RÉSUMÉ

Effet de la stérilité mâle sur la teneur en huile et le rendement en grains chez le tournesol (Helianthus annuus L.)

Des études de la teneur en huile et du rendement en grains d'hybrides expérimentaux, fabriqués à partir d'individus d'une population de tournesol, ont montré des différences entre les hybrides mâle-stériles et mâle-fertiles. Les premiers avaient une teneur en huile supérieure de 2,62 p. 100 et un rendement supérieur de 8,22 p. 100 (par rapport à des variétés témoins). Des différences ont aussi été trouvées entre des lignées mâle-stériles cytoplasmiques et leurs mainteneurs de stérilité isogéniques. En autofécondation, les mâle-stériles avaient 1,25 p. 100 d'huile en plus de 4,11 g/plante de semences en plus et, en fécondation libre, 1,13 p. 100 d'huile en plus et 7,56 g/plante de semences en plus. Il semble que les différences entre mâle-stérile et mâle-fertile ne soient pas dues au cytoplasme mâle-stérile de *Helianthus petiolaris*, ni à la fécondation libre par rapport à l'autofécondation. Aussi, est-il proposé qu'elles soient liées à l'absence de production de pollen.

Mots clés complémentaires : Hybride, lignée, autofécondation, fécondation libre, castration chimique, cytoplasme.

I. INTRODUCTION

Oil is the primary product of sunflowers and the oil content of the seed is one of the most important characters in the breeding of new lines and hybrids. Following 30 years of breeding work, notably by PUSTOVOIT, the oil content in the whole seed was increased from about 30 % to 45-50 % (DVOREADKIN, 1974). Present day varieties contain 50 to 55 % oil and we have found plants with more than 60 % oil. Oil content is a highly heritable character (FICK, 1975), determined by the genotype of the plant bearing the seed (THOMPSON *et al.*, 1979), but many other factors have notable effects, particularly temperature

(BEDOV, 1980) and self or cross pollination (LOW, 1982).

Sunflower varieties are single cross or three way hybrids, the female parents of which contain cytoplasmic male sterility (Cms) which originates from a cross between *Helianthus petiolaris* Nutt. and *H. annuus* L. (LECLERCQ, 1969). The male parents contain one or two dominant restorer genes (LECLERCQ, 1982).

Among the measurements of combining ability in a recurrent selection programme for restorer genotypes, it was noticed that the highest oil contents were always found in completely male sterile hybrids. To determine whether this was chance or whether it was

due to the male sterile cytoplasm, to the male sterility or to cross pollination, studies were made using inbred lines with or without *H. petiolaris* cytoplasm and under self, sib and open pollination. The seed yield of the different treatments were also compared. This paper reports these results and discusses possible consequences.

II. MATERIALS AND METHODS

SUNFLOWER GENOTYPES

A. Recurrent selection programme hybrids

These hybrids were made by crossing a cytoplasmic male sterile tester line with So individuals from a male fertile restorer population obtained by interpollination of 15 genotypes in an isolated plot. Although all these genotypes were homozygous for restoration, they did not all contain the same restorer genes, which led to segregation for this character. In addition, not all contained male sterile cytoplasm, with the result that some non-restorer segregants were male fertile but male sterility maintainers. Thus some hybrids had completely restored male fertility, some showed segregation and others were completely male sterile. They were tested in a series of 12 trials, all in the same field, with 21 hybrids and 4 control varieties in each trial, 2 replications and plots of 8.8 m².

B. Inbred lines

The inbred lines were bred at Clermont-Ferrand or introduced from the U.S.A. They were chosen to represent a wide range of flowering dates and degrees of self-fertility. In all cases the Cms form and its isogenic maintainer were available. For the comparison of Cms and male fertile plants under self-pollination (or sib-pollination in the case of Cms plants) four or five pairs of plants from 10 different inbred lines were used. For the comparison under open pollination between Cms, male fertile and chemically sterilized maintainer plants, eight different inbred lines (not necessarily those used for the selfing) were used, with 9 or 10 plants for each treatment. Each inbred line contributed the same number of plants to each treatment.

Sib crosses and self pollinations were carried out under paper and cellophane bags and tubes. Open pollinated plants were covered with paper bags after flowering to protect from bird damage.

C. Chemical sterilization

In order to obtain male sterile plants without *H. petiolaris* cytoplasm, the flower buds of maintainer plants were treated with 1 ml of gibberellin A₃ (Berelex, ICI-SOPRA) at a concentration of 25 ppm when they measured about 1 cm in diameter (star stage). However the effects were unsatisfactory, as male sterilization was only partly successful.

D. Oil contents

The oil content of hybrids was measured on 10 g whole seed samples collected after harvesting the whole of each plot. For inbred lines the 10 g whole seed sample was from individual plants. A Newport Nuclear Magnetic Resonance apparatus was used and results are the mean of two 32-second integrations.

III. RESULTS

The analyses of oil content in hybrids and individual plants are given in table 1. Comparison of the 22 entirely male sterile and the 62 entirely male fertile hybrids shows that they are highly significantly different, the former having a mean oil content of 50.50 %, compared with 47.88 % for the latter. Even when the 62 completely and 168 partially male fertile hybrids are grouped the difference remains : the mean of male fertile hybrids increases only to 48.50 %, still 2 % less oil than in the male sterile hybrids.

The results for the sibcrossed male sterile inbreds and their selfed maintainers are comparable although less distinct. The 49 male sterile plants had a mean of 1.25 % more oil than the 49 isogenic male fertile plants with which they were pollinated. For individual plants this difference is significant ($t = 2.80$, 96 d.f.) although it may be noted that taking the inbred line means this is not the case (table 3 : $t = 1.63$, 18 d.f.).

Under open pollination the male sterile plants had 1.13 % more oil than the male fertile plants. Again

TABLE 1
Comparison of oil contents in male sterile and male fertile hybrids and inbreds.
Comparaison des teneurs en huile chez des hybrides et des lignées mâle-stériles et mâle-fertiles.

Material	Constitution		MS/MF	Pollination	Hybrid or plant number	Mean oil content	Variance	Difference	t
	Cytoplasm	Rf							
Hybrid	Cms	Rf	MF	Open	62	47.88	1.77	} 2.00	} 2.62
	Cms	Rf + rf	MS + MF	Open	230	48.50	1.57		
	Cms	rf	MS	Open	22	50.50	1.51		
Inbred	Cms	rf	MS	Sib	49	45.30	47.39	} 1.25	} 2.80 HS
	N	rf	MF	Self	40	44.05	37.68		
	Cms	rf	MS	Open	76	49.73	25.29	} 1.13	} 3.68 HS
	N	rf	MF	Open	76	48.60	32.82		
	N	rf	MS (gb)	Open	57	42.75	10.79		

the difference between individual plants (table 1) is significant, but not that between the means of inbred lines (table 3).

Seed yields were also studied (table 2). An effect comparable to that on oil content was found. Expressed as a percentage of the yield of the control varieties the 22 male sterile hybrids had 8.22 % greater yield than the 62 entirely male fertile hybrids. There was, however, more irregularity than for oil content and the *t* was lower (2.87 for yield, 13.05 for oil, 82 d.f.). In contrast, the yields of inbred lines under self and sib or open pollination showed a clearer difference than for oil content (table 2). Under sib pollination the Cms plants produced a mean of 4.11 g per plant more seed than the maintainers, a difference of 20.50 % and under open pollination the difference was 7.76 g, 16.50 %. Under both types of pollination the differences both between individual plants and inbred line means are highly significant (tables 2 and 3).

It had been planned to determine the effect of male sterility on oil content in the absence of *H. petiolaris* cytoplasm by observing maintainer lines treated with gibberellin but this was not successful. It had been shown earlier that gibberellin treatment reduces seed yield (VEAR, 1981) and it was found that, in addition, the oil contents of the treated plants were very considerably reduced in comparison with their untreated counterparts (table 1).

IV. DISCUSSION

The results show that there was an average of 1 or 2 % more oil in the seed from male sterile plants, compared with their male fertile counterparts and that the yield of the former was 8 to 20 % higher than that of the latter. In maize differences in yield and plant height related to the presence of Texas cytoplasm have

TABLE 2

*Comparison of seed yield in male sterile and male fertile hybrids and inbreds.
Comparaison des rendements en grains chez des hybrides et des lignées mâle-stériles et mâle-fertiles.*

Material	Constitution		MS/MF	Pollination	Hybrid or plant number	Mean seed yield (g)	Variance	Difference	<i>t</i>
	Cytoplasm	Rf							
Hybrid	Cms	Rf	MF	Open	62	94.97	196.89	} 8.40	2.87 HS
	Cms	rf	MS	Open	22	103.19	246.36		
Inbred	Cms	rf	MS	Sib	49	20.05	135.81	} 4.11	6.74 HS
	N	rf	MF	Self	49	15.94	117.95		
	Cms	rf	MS	Open	80	45.93	470.03	} 7.56	5.59 HS
	N	rf	MF	Open	80	38.37	323.86		

TABLE 3

*Mean oil contents and seed yields of the male sterile and male fertile forms of the inbred lines studied.
Teneurs en huile et rendements en grains moyens chez les formes mâle-stériles et mâle-fertiles des lignées étudiées.*

Inbred	Oil content (%)				Seed yield (g)			
	Open poll.		Sib/self poll.		Open poll.		Sib/self poll.	
	MS	MF	MS	MF	MS	MF	MS	MF
AA	47.5	42.8			52.3	42.8		
BL	50.5	49.3	40.5	42.0	37.9	31.0	15.6	16.2
CAA			52.5	50.9			25.6	13.1
CAB			46.5	40.8			33.5	19.9
CAC			38.1	38.0			13.0	11.8
CD	58.9	58.7	54.7	55.3	47.6	34.4	33.3	32.3
CX			51.8	47.7			20.9	16.9
CY			46.4	44.2			6.2	4.6
CZ			46.4	46.0			11.1	7.5
DD	45.3	46.5			36.4	52.6		
DL	51.0	49.5			52.5	45.0		
ET			36.9	37.8			22.2	14.8
GH	54.7	52.8			71.4	47.0		
GN			39.2	38.0			19.0	20.8
IB	46.5	41.9			20.3	13.4		
WG	45.1	46.2			49.2	39.3		
dif	1.13		1.25		7.56		4.11	
<i>t</i>	1.62 NS		1.23 NS		2.54 HS		2.84 HS	

been observed (DUVICK, 1965 ; CAUDERON & CAENEN, 1967). However, for the present results it appears unlikely that the differences are due to the *H. petiolaris* cytoplasm, since all the hybrids from the recurrent selection programme contained this cytoplasm as they all had the same Cms line as female parent. Their male fertility or male sterility was determined by the presence or absence of restorer genes. Nevertheless, it is possible that linkage of restorer genes with low-oil genes could be responsible for the greater difference (2.62 %) in oil content between male sterile and male fertile hybrids, compared with the inbred lines (1.13 and 1.25 %). This was in contrast with the results for seed yield, where the greatest difference was observed in the inbred lines.

It has frequently been observed (LOW, 1982) that the oil content of inbred lines is higher after open-pollination than after selfing, and the present results show that seed yield is generally more than doubled (45.93 g and 20.05 g for open and self-pollination respectively). Similarly in onion, BERNINGER (1976) explained an increased seed production in male sterile plants by the absence of self-pollination. However, since the sib-crossed Cms sunflower plants, pollinated by their isogenic maintainers, and thus in theory selfed, showed higher seed yield and oil contents, the difference does not appear to be due to 100 % cross-pollination of male sterile plants compared with partial self-pollination of male fertile plants. Manual pollination cannot have had any effect either, since the transfer of pollen from male fertile to male sterile plants requires rubbing both capitula together.

Thus, since neither the type of cytoplasm nor the type of pollination appear to play important roles, it seems likely that the higher seed yield and oil content of male sterile plants are directly related to male sterility, that is the absence of pollen production. Since it was not possible to prove this by the use of

chemical sterilization, we will try to obtain further confirmation by studying male sterile and male fertile plants in lines with genes giving male sterility, although the results may not necessarily be the same since, with genic male sterility, the anthers are of almost normal size and some non viable pollen is produced (LECLERCQ, 1966). In Cms plants, pollen develops only up to the tetrad stage and anthers are very much reduced in size (VEAR, 1973). One hypothesis to explain the differences observed is that the energy not used by male sterile plants in pollen production contributes to better seed and oil production (LECLERCQ, pers. comm.).

From a practical point of view, these results must be taken into account in combining ability tests. When more or less sterile hybrids are compared with male fertile hybrids, either also under test or control varieties, allowance must be made for male sterility. One to 2 % should be subtracted from their oil content, and at least 5 % from the seed yield.

In theory, the cultivation of varieties showing only partial male fertility would give crops with higher yields and oil contents so long as there was sufficient pollen to give satisfactory seed set. However, if in a crop not all plants are fertile, and accidents would occur if the weather or location were such that bees were not active in cross pollination. Sunflowers are generally bred for self fertility, so that good yields can be obtained in the absence of bees and it appears dangerous to reverse this policy.

In conclusion, these results appear of practical importance in combining ability tests and pose an interesting fundamental problem of plant, and more particularly seed, development in relation to male sterility and its possible basis.

Reçu le 18 novembre 1983.

Accepté le 25 mai 1984.

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