T1978AGRO25

POLLINATION AND FERTILIZATION IN SUNFLOWERS

By

Low, A., M.C. Mackay, and G. Pistillo
(Australia)

Abstract

The effect of high mean and maximum temperatures on pollen viability, bee activity in cross pollination, and inherent compatibility levels are important inputs to fertilization in sunflowers.

Application of these facts enabled world-wide pollen importations to be successful, without transgressing plant variety protection acts, and a rapid build up of locally adapted germplasm pools.

Recent open pollinated and hybrid cultivars express a wide range in self-incompatibility at levels below that considered potentially desirable.

Unless adequate pollinating insects are assured, particularly honey bees, these levels are unsatisfactory support for those other inputs necessary to achieve high yields.

In order to enable two industries to prosper, it is suggested a priority be given to improve nectar abundance, concentration, and sugar balance for honey production, as well as raise crude protein content of pollen above 20%.

In this way pollination charges could be reduced and placement of hives be as common and economic as the placement of seeds and fertilizers.

Introduction

In terms of production, sunflower (Helianthus annuus) is a relatively new crop to Australia.

100,000 tonnes of seed from 170,000 hectares, of predominately raingrown crop, is an average over the period 1973-1978, which suggests there is room for research and extension.

At present it is Australia's most important oilseed, but disillusionment by farmers has been widespread. Failure to obtain expected seed yields has been a result of many factors, with lack of experience, unsuitable cultivars, insufficient moisture, and disease often being quoted.

This paper suggests that pollination and fertilization to obtain optimum seed set has been overlooked. Acceptable plant growth and head size have often been achieved but not realized in seed yields.

The more important constraints on achieving fertilization are in effecting pollination and managing sporophytic incompatibility systems. In the former, improved attractiveness, in terms of higher protein content of pollen and nectar quality, have future relevance in the economics of pollination services. The development of incompatibility was a major objective, prior to the advent of cytoplasmic male sterility, as a means of generating heterosis. However, if complete self compatibility is not to be the desirable genetical alternative, then the role of insect pollinators must remain a priority.

Heterosis, generated by inter plant crossing through honey bees (the main insect pollinator in sunflowers), leads not only to higher yields but also oil content. Additionally, germination is improved through larger seed size, and a second industry to beekeepers in honey and pollen collection should also be considered.

As areas sown to sunflowers and alternative crops increase across the world, an improvement in nectar abundance, concentration and quality merits consideration. Increasing its competitiveness to ensure cross pollination should receive a priority not less than other more publicized inputs.

Materials and Methods

To assess the duration of pollen vaibility commensurate with an anticipated world-wide transport requirement, and also in relation to the period of anthesis in open pollinated cultivars, a bulk sample of Sunfola 68/2 was exposed to three air temperatures and three desiccant treatments.

Constant temperatures of $4^{o}C$, $27^{o}C$ and $35^{o}C$ were effected over a three week period. The pollen was stored alone, mixed with silica gel, and separated from silica gel by a cotton wool plug for each temperature treatment.

Plants of the same cultivar, grown under controlled conditions at $27^{o}C$, were emasculated and pollen from the nine treatments was applied liberally to 20 florets per treatment. Replication was eightfold. After three days the numbers of shrivelled styles were recorded, followed later by seed set counts.

Field trials were conducted under gravity irrigation on ridges 100 cm apart, with intra row spacing at 10 cm. Fertilizer was applied at 100 kg N and phosphorus at 30 kg P ha^{-1}, 10 cm below the seed bed.

Prior to anthesis insecticides were usually applied to minimize possible damage. Immediately after, plants of similar physiological development were selected in close proximity to each other and treatments were applied as follows. Self pollination was effected by covering the capitulum with lightweight, waterproof paper bags, and tying these with string around the base of the capitulum. Nylon tulle nets were applied in a similar manner to prevent large insect pollination. Naturally pollinated plants were labelled at the same time. All treatments were bagged after flowering for uniformity and to prevent insect and bird damage.

Seed yields from each treatment were recorded and results for self pollination and small insect pollination were expressed as a percentage of naturally pollinated seed yield.

Results

Initial investigations at the commencement of the breeding program in 1973-74 were to assess the effect of temperature and desiccation on pollen longevity and thus fertilization, as it was intended to import pollen from overseas to build up local gene pools. In Table 1 temperature is seen to be the more important, pollen viability falling off rapidly by 35°C.

TABLE 1. Numbers of set seed out of 160 florets.

Pollen treatment	Exposure for three weeks 4°C	27°C	35°C	Mean
No desiccant	81	72	31	61
Pollen + desiccant (S)	98	76	23	66
Pollen + desiccant (M)	87	69	21	59
Mean	89	72	25	

Temperature LSD ($P < 0.01$) = 27.3
(S) Desiccant kept separate.
(M) Desiccant mixed with pollen.

As inclusion of desiccant gave no advantage and would occupy space, pollen alone was imported in self seal plastic bags enclosed in insulated postal envelopes. A 50% or greater seed set was considered satisfactory.

Shrivelled style numbers after three days did not give a good estimate of final seed set. It was observed that more styles shrivelled after the observations were made, which suggests three days to be too early.

To determine the effectiveness of natural insect pollination a glasshouse trial was undertaken with temperature being controlled at 27°C. Two hybrid and one OP cultivar were sown in pots with light, water and nutrient levels being optimum.

Pollination treatments were effected in three ways; honey bees, brush sibbing on alternate days, and selfing using paper bags. Full seed weight was recorded from 12-20 plants for each pollination X cultivar treatment. Percentage seed set is listed in Table 2.

TABLE 2. Selfed seed expressed as a percentage of sib brushed (SB) or bee pollination (BP) in three cultivars.

Cultivar	SB (%)	BP (%)
Experimental hybrid D4	60	104
Commercial hybrid Hysun 20	59	NA
Commercial Sunfola 68.2 (OP)	35	62

NA = not available

These preliminary results demonstrated that in the local open pollinated cultivar, selfing set less seed than both brush and bee polination. All cultivars responded most to brush sibbing, but D4 had sufficient self compatibility to match the level of bee pollination experienced.

In 1974-75, three cultivars, one hybrid and two OP's, were sown in the field at a plant density of 90,000 ha^{-1} to look at the effectiveness of insect pollinators. Plants were allowed to be fertilized by natural pollination (NI) or small insects and wind only (SI), with a self pollinated (SP) control. Results are shown in Table 3.

TABLE 3. Seed set of selfed and small insect treatments as a percentage of naturally pollinated yield.

Cultivar		Recommended density 90,000 plants ha^{-1}
Chernianka (OP)	SI	83
	SP	65
Difference n.s.		
Sunfola 68.2 (OP	SI	58
	SP	15
(LSD ($P < 0.05$) = 30.8		
Hysun 30 (hybrid)	SI	95
	SP	55
Difference n.s.		

None of the cultivars were satisfactory for self compatibility, especially Sunfola 68.2. Wind and small insect pollination together raised seed set which was significant with Sunfola 68.2.

Yields in the trial were still good with natural pollination averaging 3-8 t ha^{-1}. As a further measure of growth, head diameters differed between cultivars expectedly, but pollination treatments were not significantly different, Low et al (1974). This was further evidence that bagging was not a serious complication, when only during the 10 day period of anthesis bags were not common to all treatments.

After the release in 1975-76 of a series of new commercial and experimental hybrids, a compatibility check was undertaken with plant densities from 10,000 to 200,000 ha^{-1} being superimposed.

Results clearly showed cultivars varied considerably, confirming genetic control of compatibility was significant. As plant density was again insignificant, only cultivars have been presented in Table 4. In this trial a male sterile A line was included to check bagging efficiency and inter plant pollination due to wind and small insects. As no pollen was available for inter floret pollination from the same capitulum, a reduced level of seed set was recorded compared to the previous table (Table 3). Yields for the control Sunfola 68.2 were 1.64 t ha^{-1} at 75,000 plants ha^{-1}.

TABLE 4. Selfed seed weight as a percentage of natural insect pollination in nine cultivars

Cultivar	Selfed as % of NI
Open pollinated	
Sunfola 68.2	2
AO 6	9
Golden cob	17
Commercial hybrids	
Hysun 10	88
Hysun 20	44
Hysun 30	32
Suncross 51	15
Suncross 52	5
Experimental hybrids	
PEH 10	76

Cultivars LSD ($P < 0.01$) = 30.35

In 1976-77 two sowing dates were introduced to effect a temperature difference at flowering. Such a difference eas expected to influence the plant physiologically and also insect activity. Temperatures over anthesis (10 days) are listed in Table 5.

TABLE 5. Temperatures for two flowering periods.

Periods	Temperatures °C Daily Mean	Mean Maximum	Number days over 35°C
1st flowering date (15-24 Dec. 1976) (sown 29 Sept. 1976)	20.9	28.3	2
end flowering date (4-13 Feb. 1976) (sown 29 Nov. 1976)	27.8	35.0	5

In Table 6 four OP's and three hybrids are presented over both sowing dates.

Environmental differences affected some cultivars more than others, Hysun 30 and G339.13 being particularly noticeable but in different directions. The other cultivars were relatively unaffected, having either a good or poor level of compatibility for both periods.

Average seed yields for natural pollination in t at 60,000 plants ha^{-1} for Sunfola 68.2, were 2.99 and 4.67 for first and second sowing dates. The hybrid Hysun 30 gave 3.24 and 5.42, respectively.

TABLE 6. Selfed seed weight as a percentage of natural pollination in seven cultivars.

Cultivar	1st sowing date	2nd sowing date
Open pollinated		
Sunfola 68.2	3	2
Chernianka	64	53
G 339.13	30	92
Golden cob (Polestar)	7	11
Hybrids		
Hysun 10	80	88
Hysun 20	16	6
Hysun 30	52	26
	LSD (P < 0.01 = 33.61	LSD (P < 0.01) = 26.64

Although four plant densities were included there were no significant interactions, confirming earlier results.

During 1977-78 further new commercial cultivars were screened with some of those previously tested. The results in Table 7 demonstrate that higher levels of self compatibility are desirable, should insect pollination be inadequate. Yields in g were also compared in a paired t test.

TABLE 7. Selfed seed weight in g per plant as a percentage of natural pollination (NI) in 11 cultivars.

Cultivar	Selfed as % of NI	Mean difference in yield (g)	95% confidence limits
Sunfola 68.2 (control)*	4	57.8	44.5 - 71.2
Hysun 10	60	25.2	12.0 - 38.4
Hysun 21	59	22.2	8.9 - 35.5
Hysun 30	59	28.1	14.1 - 42.2
Suncross 51	47	26.7	18.8 - 34.6
Suncross 52	37	35.8	17.9 - 53.7
Suncross 53	35	37.2	22.3 - 52.0
Sungold 204	28	47.3	24.8 - 69.7
Sungold 11	42	36.7	11.8 - 61.7
Sunance	16	75.7	32.4 -118.9
Oilmaker	63	26.2	n.s.
LSD (P <0.01)	26.4	n.a.	n.a.

* Open pollinated, rest are hybrids.

Trials were carried out during 1976-77 and 1977-78 in farmers' fields. At Site A about 50 two story hives were noticed 1 km away at the rate of 1 hive ha^{-1}. At Site B, 80 hives of two stories were placed adjacent to 50 hectares.

A hybrid Hysun 30 was sown at Site A and two open pollinated cultivars VNIIMK and Sunfola 68.2 at Site B.

Transects were made across the fields leading away from the hives, pairs of plants of equal maturity being selected and bagged overy 5-10 meters.

Counts of visiting bees were made at Site B. Mean yield increases over self pollination, due to natural pollination, and 95% confidence limits for these are listed below for both sites.

(a) Site A - Hysun 30 (Hybrid) n = 62
Mean yield increase (g) 26.1
95% confidence limits (g) 21.8 - 30.4

(b) Site B - (i) VNIIMK (OP) n = 81
Mean yield increase (g) 26.6
95% confidence limits (g) 22.7 - 30.4
(ii) Sunfola 68.2 (OP) n = 34
Mean yield increase (g) 26.1
95% confidence limits (g) 21.8 - 30.4

Losses of this magnitude, when extrapolated to 50,000 plants ha^{-1}, amount to approximately 1.3 t ha^{-1} worth $A250. The hire of 1.6 hives h^{-1} at $A10 a hive or $A16 makes a significant profit.

Discussion of Results and Conclusions

An important factor affecting pollen viability and expression of self-compatibility levels are temperatures exceeding 30-35°C during anthesis (Tables 1 and 6). Application of this and the knowledge that the normal percentage of humidity in pollen was not deleterious, facilitated the world-wide collection and transportation of pollen using insulated postal packages.

The use of pollen importations rather than seed to rapidly generate new gene pools for local adaptation has been found to be successful (Low et al, 1974). Other applications, for testing new restorer and for backcrossing new maintainer lines for A line conversions, reduce the need to maintain world-wide collections.

In Table 2 maximum seed set was obtained with brush sibbing between plants for both hybrid and OP cultivars. An environment of 27°C obviated any complications which may have been contributed by high temperatures. An experimental hybrid D4 appeared to have a better level of self compatibility than the OP, Sunfola 68.2.

In a field trial recorded in Table 3, three cultivars failed to record satisfactory levels of self compatibility, the best being only slightly more than half that of natural pollination. Sunfola 68.2 was again very low in contrast to the other OP Chernianka. Small insects were largely responsible for significantly raising the seed set in Sunfola 68.2, as wind pollination would have occurred in the naturally pollinated treatment also.

Sudden infestation of small sucking insects such as Rutherglen bug (Nysius vinitor) are not uncommon at flowering in Australia. Large numbers are regarded as pests to be eradicated by insecticide. However, it appears to be conjectural whether these insects are beneficial or deleterious, and if so, at which levels of investation.

As new commercial and experimental hybrids, as well as local selections of imported OP's become available, further assessment of self compatibility levels were merited. In Table 4 genetic control over a wide range was evident, Hysun 10 (commerical) and PEH 10 (experimental) hybrids reaching more acceptable levels. Inclusion of a male sterile A line to check bagging excluded insect visitation at anthesis was confirmed. Covering the capitulum with tulle netting suggested that inter plant pollination by wind and small insects was minimal at only 4% of natural pollination. These two agents were therefore more effective in inter floret pollination within a capitulum, as suggested by results recorded in Table 3 with male fertile cultivars.

A range of sowing dates have been practiced by farmers to fit in with seasons or rotations, and to avoid predisposition to brown rust attack (Puccinia helianthi) with susceptible cultivars. Lower temperatures during maturation are also sought to enhance linoleic fatty acid ratios. In Table 6 two distinct sowing dates were imposed, sowing several hybrid and OP cultivars on both occasions.

In Table 5 different temperature levels were achieved and their effect was associated with a variable response to levels of self compatibility.

A Griffith selection for higher compatibility in an OP (G 669.13) responded to higher temperatures, but in a commercial hybrid (Hysun 30) it had the reverse effect. This has implications in plant breeding to match commercial sowing practices. Other cultivars both hybrids and OP's were temperature insensitive, and maintained either low, medium, or high levels of self compatibility.

As many new releases of cultivars occurred in 1977, these were checked when sown at the recommended sowing date for this region of NSW, Australia. In Table 7 it is clearly demonstrated that higher levels of self compatibility are desirable unless pollinating insects are known to be adequate.

Losses in yield, compared with natural pollination, were highly significant ($P < 0.05$). Honey bees were the major pollinating insect and were observed at 2-3 bee visits per capitulum of 2000 florets per minute before noon, during the 10 days of anthesis.

As a final study, two farmers' trials were designed to assess the effect of local and introduced pollinating insects on one hybrid and open pollinated crop. Different ratings of introduced hives of honey bees returned similar results. Perhaps the higher bee population on the OP's offset the higher level of self compatibility of the hybrid.

Increases in yield at both sites of 1.3 t ha^{-1} were highly significant (P 0.05). This appears to justify the economics of placing hives in these two instances for both the hybrid and OP cultivars at current pollination charges.

General Discussion

Papers presented at the last two world conferences, connected with the development of the plant after anthesis, are only about 20% of the total including more recent interest in fertility restoration for hybrid production. Has hybrid technology, however, generated a complacency in regard to levels of self compatibility? Prior to cytoplasmic male sterility, breeding self incompatiblity to exploit hybrid vigor was premeditated. Then the role of pollinating insects was an essential input, but it is argued this priority remains in Australia.

Technically, cross pollination remains undisputed in all cultivars, the economics of imported honey bees only being queried. Increases in yield, as demonstrated in this paper, warrant a priority at least equal to any other investigation. Due to genotypic differences in self compatibility in both hybrids and OP's, and their interaction with temperature, how valid are national cultivar trials? If localized levels of pollinating insects are a vital source of variation, then saturation with imported honey bees must be a feature if genotypic potential is to be measured.

Other pollinating insects should not reduce the necessity for honey bees, as only the latter can be multiplied and controlled, and have only a beneficial role. However, due to competitive crops and natural flora, attempts should be made to increase the attractiveness of sunflowers to improve pollen protein for brood raising, and nectar for pollination and honey quality.

References

Abstracts of the 7th International Sunflower Conference, June 27-July 3, 1976, Krasnodar, USSR.

LOW, A., G. PISTILLO, and M.C. MACKAY, 1974. Pollination A Rep. Div. of Irrig. Res. CSIRO Aust. 1974-75.

Proceedings of the 6th International Sunflower Conference, 22-24 July 1974, Bucharest, Romania.