Can the intraplant pollen offer be related with fruit set failures in sunflower (*Helianthus annuus* L.)? Studies in modern hybrids and cross-pollinated varieties

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ABSTRACT

- *Background and aims*: Sunflower plants, even grown under optimal culture conditions, always present seedless or incomplete developed fruits (IDF), mainly in the central regions of the capitulum. Our objective was to identify, qualitatively and quantitatively, the intraplant pollen offer and its possible relationship with the generation of IDF.

- *Methods*: Sunflower plants were grown under irrigation and fertilization in the Agronomy Dept.-UNSur (Bahía Blanca, Argentina: 38º 45' S., 62º 11' W.) in a factorial combination of two commercial hybrids and two planting dates (PD). Two cross-pollinated varieties of sunflower (Hopi and Havasupai) were also planted. Measurements were done taking into account three capitulum sectors each one equal to 1/3 of the capitulum radius (external [ES], middle [MS] and internal [IS]). Close to anthesis, pollen offer was estimated by counting pollen grains per flower (P/F). To determine the anther size in each capitulum sector, anther length (AL) and anther width (AW) were measured. The same procedure was done with the two cross-pollinated varieties. In both hybrids and for each capitulum sector the anther volume (AV), and the pollen grain volume (PV) were calculated. Also, postanthesis pollen viability was assessed using Alexander's stain. At harvest, fully developed fruits (FDF) and IDF per sector were counted.

- *Key results*: Pollen viability of the two hybrids in the two PD was not significantly different between capitulum sectors. P/F was significantly higher in the capitulum's IS compared with the MS and ES. Cross-pollinated varieties showed the same results but with no difference between ES and MS. PV did not show significant differences between capitulum sectors. AL, AW and AV were higher in the IS while the ES and the MS had intermediate values. In all cases the higher number of FDF was observed in the ES and MS and the number of IDF was generally higher in the IS.

- *Conclusions*: Even though it is not known whether failure of pollen germination on the stigmata could occur, we can conclude that the formation of IDF in different regions of the capitulum would not depend on intraplant pollen offer as both, pollen offer and viability were not limiting. The intra inflorescence positive centripetal gradient of pollen quantity observed in the plant material used in this work may respond to a legacy of the species as a strategy to invest resources in the sporophytic generation during the late stages of anthesis. So, the flowers of the ES, which open first, should have a greater chance of allogamous pollination, while flowers opening up later (IS), because of temporary, positional and morphological disadvantages, would be forced to self-pollination. This response could avoid photoassimilate supply competition between proximal and distal regions of the capitulum.

- *Nature of the contribution to current knowledge achieved by this research*: An important finding emerging from the results of this work is the identification a positive centripetal gradient of pollen grain number per flower within the capitulum of both hybrids and two cross-pollinated varieties. This disagrees with the quantitative description of pollen production in other species with racemose inflorescences. Results from the present work, regarding the fact that early-domesticated genotypes, Hopi and Havasapui, showed the same quantitative pollen pattern, suggests that this character would be a characteristic of the genus and that under certain growth conditions, it could be expressed as it has been described here.

Key words*:* *Helianthus annuus* L.; pollen offer; pollen viability; crop yield.

INTRODUCTION

At maturity, capitula of self-sterile or self-fertile sunflower cultivars usually show a set of fruits with a different degree of pericarp and embryo development. In most of them, the embryo reaches its full size filling the internal cavity of the ovary. These fruits can be defined as "fully developed" (FDF) (Lindström *et al*., 2004). Nevertheless many mature fruits often contain ovules that did not fully develop into seeds. In these fruits, seed growth processes stop at different moments or never occurs, leaving the fruit with an incompletely developed pericarp and/or seed (seedless, "incompletely developed" fruits or IDF; Lindström *et al*., 2004).

Failures in fertilization and physiological or morphological defects in the ovary and embryo or vascular deficiencies at the ovary-receptacle interphase (Hernández and Orioli, 1991; Alkio and Grimm, 2003) either post-pollination or post-fertilization, genotype- and/or ambient-induced, are most common reasons for the presence of IDF in sunflower (DeGrandi-Hoffman and Chambers, 2006). Poor seed set or unfilled fruits can occur also due to inadequate pollination (Hernández, 2008). A detailed study of visit paths patterns of diurnal pollinators, mainly honeybees (*Apis mellifera* L.), in sunflower capitula, (Hernández, 2008), showed a significant negative correlation ($r=-0.61$; $p<0.05$) between the areas covered by the foraging paths and the IDF.

Because the development of IDF is a cause of important reductions in seed and oil production in sunflower, determining the reasons and the degree of this reduced fecundity are important goals in studies of sunflower management and breeding.

The aim of the present work was to determine qualitatively and quantitatively the intraplant pollen offer and IDF generation. High oleic hybrids were used, since these genotypes have recently been incorporated into the value chain of sunflower production and presents relevant importance for the future sunflower production in Argentina.

MATERIALS Y METHODS

The experiment was carried out at the Agronomy Dept. UNSur, Argentina (38º45' S, 62º11' W). Two high oleic sunflower hybrids: Dekasol Oilplus 3845 (HA) and Dekasol Oilplus 3945 (HB), were sown in two planting dates, October 25 and November 25, in 2010. Also two cross-pollinated sunflowers, Havasupai (HAVA) and Hopi (HOPI) were sown on October 14, 2010. After seedling emergence, plant density was adjusted to 5.6 plants/m^2 by manual thinning.

The experimental design consisted of completely randomized split plots, with hybrid assigned to main plots and planting date to subplots, with three replicates per treatment. Each subplot had three rows 0.70 m apart and 6.0 m long.

During the growing season, soil moisture was kept near field capacity by drip irrigation. Weeds were controlled manually and at V4 (Schneiter and Miller, 1981) the crop was fertilized with 50.0 Kg N/ha using NH_4NO_3.

Qualitative and quantitative pollen analysis

Three plants per replicate were used for measurements. Qualitative and quantitative pollen analysis were done in three capitulum sectors, each one equal to 1/3 of the capitulum radius (external [ES], middle [MS] and internal [IS]). Pollen grains per flower (P/F) were counted on 5 closed flowers from each sector. Counting was made using a haemocytometer (Godini, 1979). Anther length (AL) and width (AW) were measured in each flower used for pollen counting. Anther volume (AV) was calculated using the equation of an ellipsoid of revolution. Pollen grain diameter was measured in samples of 60 grains taken from flowers of each capitulum sector. Grain volume was then calculated using the formula of the sphere. Pollen viability was determined using Alexander's stain (1969).

Yield analysis

At harvest capitula were split by sectors (IS, MS and ES). The fruits were counted and classified into two categories: fully developed fruits (FDF) and incompletely developed fruits (IDF).

Three-way ANOVA (Infostat statistical software, 2010) was used to analyze all data in hybrids. Probabilities equal to or less than 0.05 were considered significant for main effects and interactions. The least significant difference (LSD) test was used to separate differences between planting dates, hybrids, capitulum sectors and its interactions. In the case of cross-pollinated varieties one-way ANOVA was used to assess differences between capitulum sectors.

RESULTS

There were no interactions (p>0.05) between the factors studied in any of the variables. Pollen viability was slightly different between planting dates and capitulum sectors (Table 1 and 2).

In both hybrids, pollen grains per flower (P/F) showed significant differences (p<0.05) among the capitulum sectors. The IS showed the highest value (47567 P/F), and the ES the lowest (29792 P/F); the MS showed intermediate values (40576 P/F) (Fig. 1A). Also, no significant differences (p=0.18) were observed in P/F in capitula of HAVA. In HOPI, flowers from MS and IS produced a larger amount of pollen grains than those from ES (Fig. 1B).

Pollen volume in both hybrids did not show significant differences (p=0.38) among capitulum sectors (Table 3).

Anther length, width and volume in both hybrids showed significant differences (p<0.05) among sectors, with the lowest values of the three variables in the ES and the highest in the IS (Table 3). In cross-pollinated varieties, only significant differences (p<0.05) were found in the anther length of Hopi, with the highest values (6.1mm) for IS and MS, and the lowest for ES (5.9 mm). While HAVA did not show significant differences (p>0.05) in any of the anther size parameters (Table 4).

In both hybrids, the number of FDF showed significant differences (p<0.05) among capitulum sectors, with the highest value of FDF in the ES (598), followed by the MS (456) and the IS (358) (Table 5). The IDF showed significant differences (p<0.05) between planting dates, with a higher number of IDF in the second PD than in the first one (Table 6). Significant differences (p<0.05) were also found among the capitulum sectors, with a higher number of IDF in IS (132), than in MS (83) and ES (62); no significant differences were found between MS and ES (Table 5).

Table 1. Pollen Viability (%) for each planting date, on average for hybrids and capitulum sectors. Values followed by different letters are significantly different (p<0.05).

Planting date	Pollen Viability (%)
First (October 25, 2010)	98.0 a
Second (November 25, 2010)	99.0 b

Table 2. Pollen Viability (%) for each capitulum sectors, on average for hybrids and planting dates. Values followed by different letters are significantly different (p<0.05).

Capitulum Sector	Pollen Viability (%)
External	99.1 b
Middle	97.7 a
Internal	98.7 ab

Table 3. Anther length (AL), anther width (AW), anther volume (AV) and pollen volume (PV) for each capitulum sectors, on average for hybrids and planting dates. Values followed by different letters are significantly different (p<0.05).

Sectors	AL (mm)	AW (mm)	AV (mm^3)	PV (μm^3)
External	4.9 a	0.6 a	0.9 a	22463a
Middle	5.2 b	0.7 b	1.0 b	23032a
Internal	5.3 c	0.8 c	1.1 c	21010a

Table 4. Anther length (AL) and width (AW), in mm; anther volume (AV) in mm^3, for each capitulum sector, in both cross-pollinated varieties: Havasupai (HAVA) and Hopi (HOPI). Values followed by different letters are significantly different (p<0.05).

Sectors	HAVA			HOPI		
	AL	AW	AV	AL	AW	AV
External	5.6 a	0.7 a	1.4 a	5.9 a	0.7 a	1.4 a
Middle	5.7 a	0.8 a	1.4 a	6.1 b	0.7 a	1.4 a
Internal	5.7 a	0.8 a	1.5 a	6.1 b	0.8 a	1.5 a

DISCUSSION

The existence of a positive centripetal gradient of pollen grain number per flower in capitula of the genotypes studied constitutes a remarkable finding. Anther length and width showed a direct relationship with the pollen grain counting. The IS showed the largest anther length and width, followed by the MS and ES. No significant differences were observed in pollen grain volume in all capitula positions (sectors). However anther volume showed significant differences, with higher values for the internal sector (25%) than those for the external sector. This was positively related to the number of pollen grains per flower produced in each sector, which was 27% higher in the flowers of the IS than in those of the ES.

Table 5. Number of fully developed fruits (FDF) and incompletely developed fruits (IDF) for each capitulum sector, on average for hybrids and planting dates. Values followed by different letters are significantly different (p<0.05).

Sectors	FDF	IDF
External	598 c	62 a
Middle	456 b	83 a
Internal	358 a	132 b

Table 6. Number of Incompletely developed fruits (IDF) for each planting date, on average for hybrids and capitulum sectors. Values followed by different letters are significantly different (p<0.05).

Planting Date	IDF
First (October 25, 2010)	76 a
Second (November 25, 2010)	109 b

This results have not been previously described for the Compositae in general or for the genus in particular, since it is always assumed that the amount of pollen in inflorescences declines centripetally (Buide, 2008). However, some evidence indicates that this gradient can be generated as the result of:

Gibberellins concentration. Duca (2006), states that at flowering GA_3 concentration in the sunflower capitulum declines towards its center. Since GA_3 arrests microsporogenesis (Duca *et al*., 2008, Kumar and Srivastava, 2009), so it could acts as a positional control for pollen grain production.

Architectural effect and anthesis temporal dynamic rather than available resources. Peripheral flowers (1/3 to 1/2 of the capitulum radius) are more likely to receive external pollen; those that open later (internal 1/3 of the capitulum radius) mostly will receive pollen of the same plant (Buide, 2004; 2008). Also, in species with racemose inflorescences, distal flowers, those that open later, are typically smaller and produce less and smaller ovules, fruits and seeds, but they may have larger stigmas and produce larger amount of pollen (Ashman and Hitchens, 2000).

In the primitive-domesticated *Helianthus annuus* L. lines (Hopi and Havasupai; Heiser, 1951) the same quantitative intra-capitulum pattern was observed. Could it be a genus character? In this sense, a positive centripetal gradient of the intra-inflorescence pollen production could be considered as an adaptive response to the time sequence of the anthesis of the species to ensure a homogeneous pollination of the capitulum. Thus, flowers of the ES, that open first, are more likely to be allogamous while those that open later (IS), would be forced to autogamy.

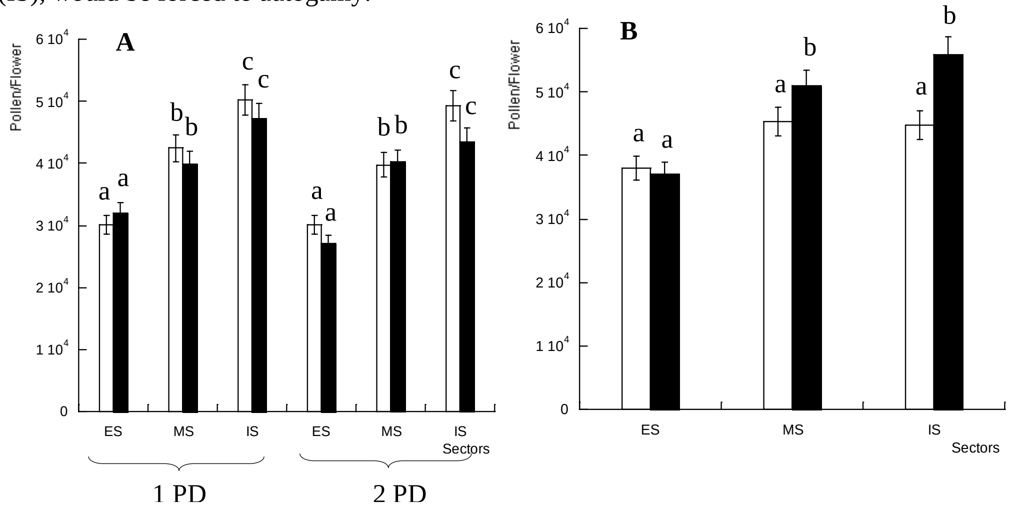

Figure 1. Pollen grains per flower in different capitulum sectors. **A**: Hybrids Dekasol Oilplus 3845 (□) and Dekasol Oilplus 3945 (■). **B**: Cross-pollinated varieties: Havasupai (□) and Hopi (■).Values followed by different letters, for the same hybrid, cross-pollinated variety and planting date (PD), are significantly different (p<0.05). ES=external sector; MS=middle sector; IS=internal sector. 1PD and 2PD: 1st and 2nd planting dates.

In some sunflower lines there is evidence of a negative centripetal gradient of pollen viability. In some short statured sunflowers, pollen viability ranges from 60-80% in the peripheral flowers to less than 5% in the internal ones (Dr. C. Sala, Nidera Arg., pers. comm.). According to the results shown here, we can infer that when lower the viability, is higher the need of pollen grains to compensate for the lack of capacity to provide male gametes during fertilization. It is known that in the sunflower capitulum the time to complete microsporogenesis is shorter in the anthers of central flowers than in peripheral ones (Dr. C. Sala, Nidera Arg., pers. comm.).

All the stated above would add to the scenario presented in the sunflower capitulum upon completion of anthesis, in agreement with the pollen compensation mechanism previously mentioned. Figure 2 illustrates an hypothesis according to the four variables described above: pollen viability, gibberellin concentration, rate of microsporogenesis and pollen grain number per flower. Only the latter one has been demostrated in the present work.

Finally, higher pollen offer of the internal flowers, as a reward factor for the pollinators, deserves a special attention. It is clear that when in the central region of the capitulum anthesis is taking place, the external flowers have been already fertilized and their ovaries are in active development. Therefore, due to a temporal and positional advantage, these flowers have already “won” the competition for resources when the central ones are opening. In this sense a higher pollen production in central flowers of the inflorescence could be an attractive mechanism for pollinators.

This intra-inflorescence variation in the amount of pollen per flower observed in the present work has been apparent in plants with specialized reproductive systems, mainly monoecious species, in which male and female functions are in different flowers. However, in species with inflorescences with hermaphrodite flowers, considerable variation can occur in the energy investment particularly focused to build a better display or attractive structures (petal size, nectar volume), biomass distribution and reproductive potential (Stephenson, 1981; Diggle, 1995). Although the average energy investment for the production of pollen is higher than that required to produce ovules (190 Cal vs. 133 Cal respectively; Smith and Evenson, 1978), in the case of the sunflower, (uniovulate flowers), this could not be an issue.

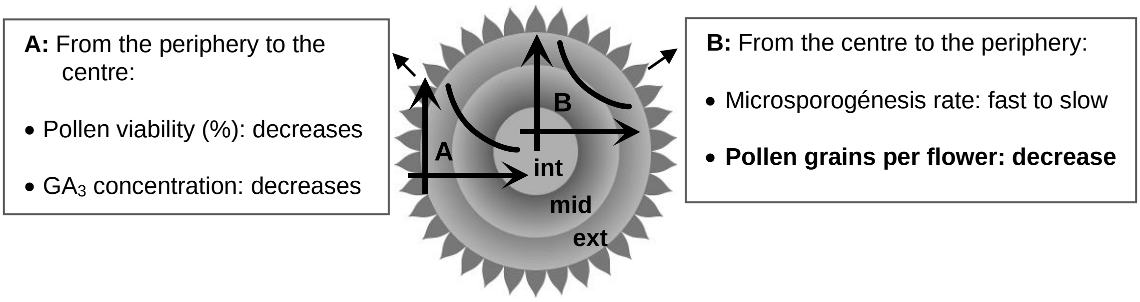

Figure 2. Schematic representation of a sunflower capitulum showing the proposed dynamics of the four variables described in this work, along the capitulum radius from the periphery to its centre, in order to explain the positive centripetal quantitative gradient of pollen grains per flower found here.

Finally, regarding sectorial grain yield in the capitulum, the largest number of IDF was produced in the IS, in spite of having the largest amount of pollen grains per flower with high viability. This result suggest that at least under the conditions in which the experiments were conducted, neither the amount nor the quality of the pollen would limit the sunflower yield. We conclude that the formation of IDF in different regions of the capitulum would not depend on pollen intra-plant offer as both, pollen quantity and quality were, in our case, not limiting. Differences in the yield potential are based on the effect of other physiological variables which have not been studied in the present work.

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