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Impact of pollination on sunflower yield: Is pollen amount or pollen quality what matters?

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ABSTRACT

Local and international information on sunflower pollination indicate that crop yield may be limited by the quality of the pollination service. This work focuses on the response of commercially-grown sunflower hybrids to variations in the amount and quality of the pollen delivered to stigmas, measured in terms of grain set, unit grain weight, grain oil concentration, and oil yield per plant as a way to assess the importance of entomophilous pollination for this crop. Three consecutive experiments including 2–8 experimental treatments each were conducted between 2004 and 2007 in Argentina. The results (a) demonstrate for the first time that the pollen amount and pollen quality that arrive to the stigma have a joint effect on sunflower grain set, (b) suggest that some yield crop components could be sensitive to this joint effect, and (c) remark the probable positive effect of the pollen redistribution across the head. Overall, results underline the importance of pollinators for the crop, and point out to possible mechanisms that explain their beneficial effect.

1. Introduction

Insufficient pollination often limits seed production in plants, sometimes severely (Burd, 1994; Larson and Barret, 2000; Ashman et al., 2004). Pollen limitation occurs when plants produce fewer fruits and/or seeds than they would with adequate pollen receipt. Whereas theory predicts that pollen augmentation should not increase seed production, numerous empirical studies report significant and strong pollen limitation in plants (Knight et al., 2005). Aizen and Harder (2007) consider pollen limitation as the joint effect of quantity and quality limitation, pointing out that quantity limitation probably occurs much less often than has been inferred from pollen-supplementation experiments, and suggesting that an expanded perspective that recognizes the fecundity consequences of pollination with poor-quality pollen would improve the ecological understanding of pollen limitation.

Most of the *Helianthus* (sunflower) wild species are naturally self-incompatible and obligate outcrossers (Heiser et al., 1969; Hurd et al., 1980), therefore the transfer of pollen between plants is necessary for fructification. A sporophytic incompatibility system contribute to the high levels of outcrossing present in wild sunflower, open-pollinated cultivars and some inbred lines (Putt, 1941; Habura, 1957; Free and Simpson, 1964; Fernández-Martínez and Knowles, 1978). In addition the florets of sunflower heads, even when they are perfect, present two mechanisms which are counter to automatic self-pollination: protandry and secondary pollen presentation (Leins and Erbar, 1989; Seiler, 1997). Therefore sunflower requires insect visits to transfer pollen from male phase florets to female phase inside a single head (capitulum).

Breeders have made efforts to develop self-fertile sunflowers, obtaining hybrids which display high levels of auto-compatibility (80–90%) (Fick and Zimmer, 1976; Vranceanu et al., 1988) and that reportedly are free of pollinator dependency. However, experimental work show that sunflower hybrids benefit from the cross pollination that occurs when insects (especially honey bees) are present in the fields (Parker, 1981; Griffiths and Erickson, 1983; Jones, 1988; Virupakshappa et al., 1992; Aslan and Yavuksuz, 2010). Presence of pollinators both increase grain set (Fick, 1979; Robinson, 1980; Krause and Wilson, 1981; Langridge and Goodman, 1981; Freund and Furgala, 1982; Low and Pistillo, 1986; DeGrandi-Hoffman and Watkins, 2000; Singh et al., 2000; DeGrandi-Hoffman and Chambers, 2006; Chambó et al., 2011) and oil concentration in the grain (Furgala et al., 1979; Mahmood and Furgala, 1983; Kleinschmidt, 1986; Calmasur and Ozbek, 1999; Kasina et al., 2007). Research carried out in Argentina generally confirmed these results (Iglesias, 1984; Lorenzatti de Diez, 1986; Ryan and Brugnoni, 1986;

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Table 1
Sunflower materials used as experimental plants and for borders and their self compatibility levels.

Experiment	Year	Experimental plants (*border plants*)
ExpA	2004–2005	Paraíso 30[b] and Contiflor 17[c] (*HA89 line*)
ExpB	2005–2006	Dekasol 4050[a] and a male-sterile line (*Dekasol 4050 and Paraíso 30*)
ExpC	2006–2007	Dekasol 4050[a] (*Dekasol 4050 and Paraíso 30*)

[a] Good.
[b] Very good.
[c] Excellent level of self-compatibility according to staff of Venado Tuerto experimental station, Instituto Nacional de Tecnología Agropecuaria, Argentina (pers. comm. to A. M. Chamer).

Amaro, 1987; González, 1994; but see Astiz et al., 2011; Chamer, 2012).

Perhaps because of the inherent complexity of the factors involved, to date it is unclear whether pollinators enhance sunflower yield by increasing the amount of pollen deposited on the stigmas, by increasing the quality of the deposited pollen, or both. Briefly, the amount of co-specific pollen grains deposited on the stigma depends on (a) the number of pollinator visits received by a floret during a certain period of time (visit rate), and (b) the proportion of sunflower pollen in the pollen loads carried by the pollinators (Dafni et al., 2005). On the other hand, since pollen grains are genetically different both within and between hybrids, pollen quality depends on the proportions of pollen from (a) the same floret, (b) another floret of the same head, and (c) another sunflower plant, which are present in the stigmatic load. Those proportions are expected to vary with the relative abundance of the insect species present in the pollinator assemblage, because more active species will be prone to transport pollen between plants, and the least mobile ones will tend to redistribute pollen within the same sunflower head.

Surprisingly, the size of stigmatic loads in sunflower has apparently never been studied in relation to yield. We are aware of a single record of the size of stigmatic loads in a field-grown sunflower hybrid, in a study unrelated to yield determination (Torretta, 2007). The effect of pollen quality has been only studied in open-pollinated cultivars, which nowadays make up just a little portion of the world's sunflower cultivated area. Free and Simpson (1964) enclosed some sunflower heads singly in muslin bags and other in pairs. Heads enclosed singly with bees had 24% of seed set, but those in pairs with bees had 45%, indicating that although sunflowers are not self-sterile, greater seed production is likely to occur following cross-pollination between florets of different heads than between florets of the same head. Hsieh (1973) obtained 72–83% of seed set from hand cross-pollination vs. 10–35% from hand self-pollination. Low and Pistillo (1986) obtained 58% of seed set from manually cross pollination vs. 22% from excluded but otherwise unmanipulated sunflower heads.

This work will focus on the response of commercially-grown sunflower hybrids to variations in the amount and quality of the pollen delivered to stigmas, measured in terms of grain set, unit grain weight, grain oil concentration, and oil yield per plant as a way to assess the importance of entomophilous pollination for this crop in Argentina. Specifically, we tested the hypothesis that pollen amount and/or pollen quality that arrives to the stigmas influence sunflower yield.

2. Materials and methods

2.1. Crop management and general experimental conditions

Three consecutive experiments were performed at the Facultad de Agronomía, Universidad de Buenos Aires (34°35′S, 58°29′W) during the summers of 2004–2005 (experiment A, hereafter ExpA), 2005–2006 (ExpB), and 2006–2007 (ExpC). Sunflower seeds of three hybrids and a male-sterile line were provided by Advanta Argentina (Balcarce, Argentina) (Table 1). These hybrids differed in their levels of self-compatibility (Table 1). Seeds were sown on 19 November 2004 (ExpA), 25 November 2005 (ExpB), and 8 December 2006 (ExpC) on a silty clay loam soil (typical Argiudol) (ExpA) or in 30 L plastic pots that were placed outdoors and filled with a 3:1 mixture (v:v) of fertilized soil and perlite (ExpB and ExpC). Three seeds were sown in each sowing point (ExpA) or in each pot (ExpB and ExpC), and plant density was later adjusted to 5.1 plants m^{-2}, with a inter-row spacing of 0.7 m. Arrays of 120 (ExpA), 72 (ExpB) and 144 (ExpC) experimental plants were surrounded by two border rows sown at the same date as experimental plants (ExpA) or a week before (ExpB and ExpC, in which border plants would be used as pollen source). In all experiments the crop was protected against weeds, birds, insects and diseases as needed. At V4 stage (Schneiter and Miller, 1981) plants were thinned to one centrally located seedling. Following Rondanini et al. (2003) the plants were watered thrice daily by a drip irrigation system using amounts of water that were periodically adjusted to meet seasonal variations in evaporative demand, and plants were fertilized with a total dressing of 3 g N per plant as $Ca(NO_3)_2$ at 25 days after emergence. Preventive applications of fungicides and insecticides were made periodically. The experiments were laid out in a randomized complete design without sub-sampling, in which homogeneity for every experimental unit (each sunflower head) was assumed.

Environmental conditions prevailing during the experiments are resumed in Table 2. Rainfall varied among experiments (data not shown), but possible differential effects are thought to have been neutralized by the drip irrigation system used. The temperature regime was similar among experimental years, thus crop developmental phases showed only minimal differences between experiments (6 days at the most). Daily incident radiation was also similar among experiments for the E–A and A phases. However, daily incident radiation during the A-PM phase was ca. 15% lower in ExpC as compared to ExpA and ExpB.

Plants were randomly assigned to experimental treatments. Number of treatment levels varied among experiments, and number of replicates per treatment varied also because some plants failed to complete head development (Table 3). All plants were bagged with disposable mob caps before start of anthesis. Mob caps were briefly (<5 min) removed only when a manipulative treatment was scheduled, which always occurred near midday (1200–1600). As heads expanded during the flowering period, mob caps were periodically repositioned to avoid contact with disk florets. Because of mob cap porosity and transparency, possible differences between bagged and unbagged heads in temperature and light availability are not expected to have significantly affected grain set. When anthesis was completed mob caps were replaced by polyester or polyamide cloth bags, which were kept on heads until harvest to prevent damage by birds.

Hand pollination proceeded in two ways. In ExpA, to simulate within-head pollen movement by insects, the target area of the head was gently rubbed with an applicator provided with a 2 × 5 cm velvet band (Fig. 1), causing the redistribution of the locally available pollen (the velvet band was replaced by a fresh one after each

Table 2
Mean environmental data and duration of the developmental phases emergence–anthesis (E–A), anthesis (A) and anthesis–physiological maturity (A–PM) for three experiments. Developmental phase E–A is the time elapsed between emergence and 7 days before full anthesis; A represents a 15-day interval centered on the mean date of full anthesis, and A–PM is the interval between 7 days after full anthesis and the R9 [i.e. the time when bracts and the back of the sunflower head become yellow and brown, Schneiter y Miller (1981)]. Values are minimum (T_{min}) and maximum temperature (T_{max}) and daily incident radiation (Rad).

Experiment	Crop developmental phase	T_{min} (°C)	T_{max} (°C)	Rad (Mj m^{-2})
ExpA (2004/05)				
Sowing date November 19th	E–A	20.0	29.7	20.0
	A	17.8	29.0	21.9
	A–PM	20.0	29.0	18.0
ExpB (2005/06)				
Sowing date November 25th	E–A	23.5	28.8	22.1
	A	24.3	29.1	21.5
	A–PM	21.3	28.3	18.0
ExpC (2006/07)				
Sowing date December 8th	E–A	20.1	30.0	21.5
	A	20.3	31.0	19.7
	A–PM	18.3	27.0	15.1

Table 3
Summary of treatments and experimental materials used in all three experiments. In ExpA and ExpB the number of replications per treatment varies because some experimental plants failed to complete development. For treatment codes see text.

Experiment	Experimental materials	Heads per treatment										
		S	HS	W	B	E	WL	WH	BL	BH	C	ME
ExpA	Paraíso 30 (hereafter P30)	10	9	9	10	–	–	–	–	–	–	–
	Contiflor 17 (hereafter CF17)	11	12	12	11	–	–	–	–	–	–	–
ExpB	Dekasol 4050 (hereafter DK4050)	–	–	–	–	9	9	9	9	9	–	–
	Male-sterile line	–	–	–	–	–	–	–	9	9	9	–
ExpC	DK4050	17	–	–	–	13	13	15	12	10	–	13

use). To simulate between-heads pollen movement, the applicator was first 'charged' with pollen from another head (from the same or a different hybrid, depending on treatment), and then rubbed on the target head. Previous tests using the applicator described and stained heads as a source of pollen, displayed ca. 12% of stained pollen over stigmas of target heads.

In ExpB and ExpC, to possibly remove the potentially confusing effect of self pollen in cross-pollinations, target florets were previously emasculated. Heads were uncovered very early in the morning (0600–0700), when anthers were still undehisced and stigmas had not started to elongate, and anther tubes of recently opened florets were carefully removed with fine forceps [in sunflower 1–4 circles of disk flowers open daily for 5–16 days (McGregor, 1976)]. Overall, ca. 18,000 florets were emasculated. Pollen accidentally deposited on stigmas was washed off with a water spray, and heads were rebagged. Microscopic inspection of a subsample of stigmas from emasculated florets showed an average of 198 pollen grains per stigma ($n = 20$ stigmas collected on 2 different days) while without emasculation open-pollinated florets reportedly show ca. 900 pollen grains per stigma (Torretta, 2007). Emasculated florets were later pollinated by being gently rubbed with a small artist brush, which was previously 'charged' by picking a small pollen amount from a Petri dish. This pollen was previously collected from designed heads and stored in Eppendorf tubes at 4 C (pollen viability was 87–99%, DAB test for peroxidases, Rodríguez-Riaño and Dafni, 2007). To create low- and high-pollen amount conditions, the Petri dish was provided with either one or two pollen spoonfuls with a small lab spatula. On average, stigmas pollinated at these doses displayed 661 and 1272 pollen grains, respectively ($n = 8$ stigmas per treatment).

Floral phenology of experimental plants was checked against published information (e.g. Seiler, 1997). In particular, stigmatic receptivity was assessed with the Peroxtesmo test (Dafni and Maués, 1998).

2.2. Experiment A

It was intended to test the effects of pollen quality on yield components. The four treatments included: Automatic self-pollination (S): heads were bagged and left unmanipulated until harvest; Hand self-pollination (HS): daily, bagged heads were briefly uncovered and the area with open florets was gently rubbed with an applicator as described before (Fig. 1), thus redistributing the locally available pollen; Within-hybrid hand cross pollination (W): as treatment

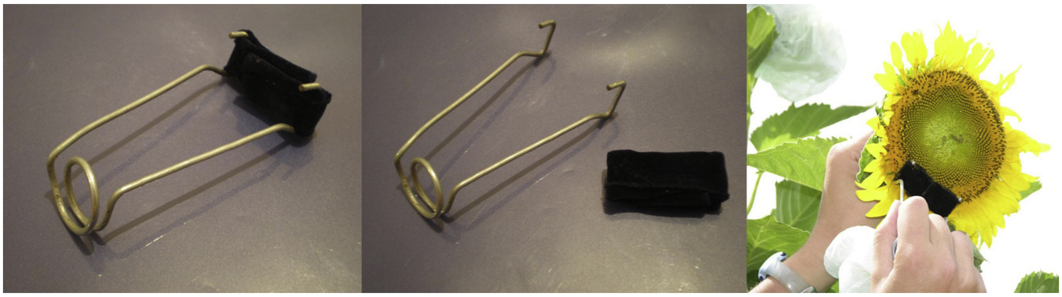

Fig. 1. Applicator used in Experiment A. Complete device (left), with velvet band removed (center), at work (right).

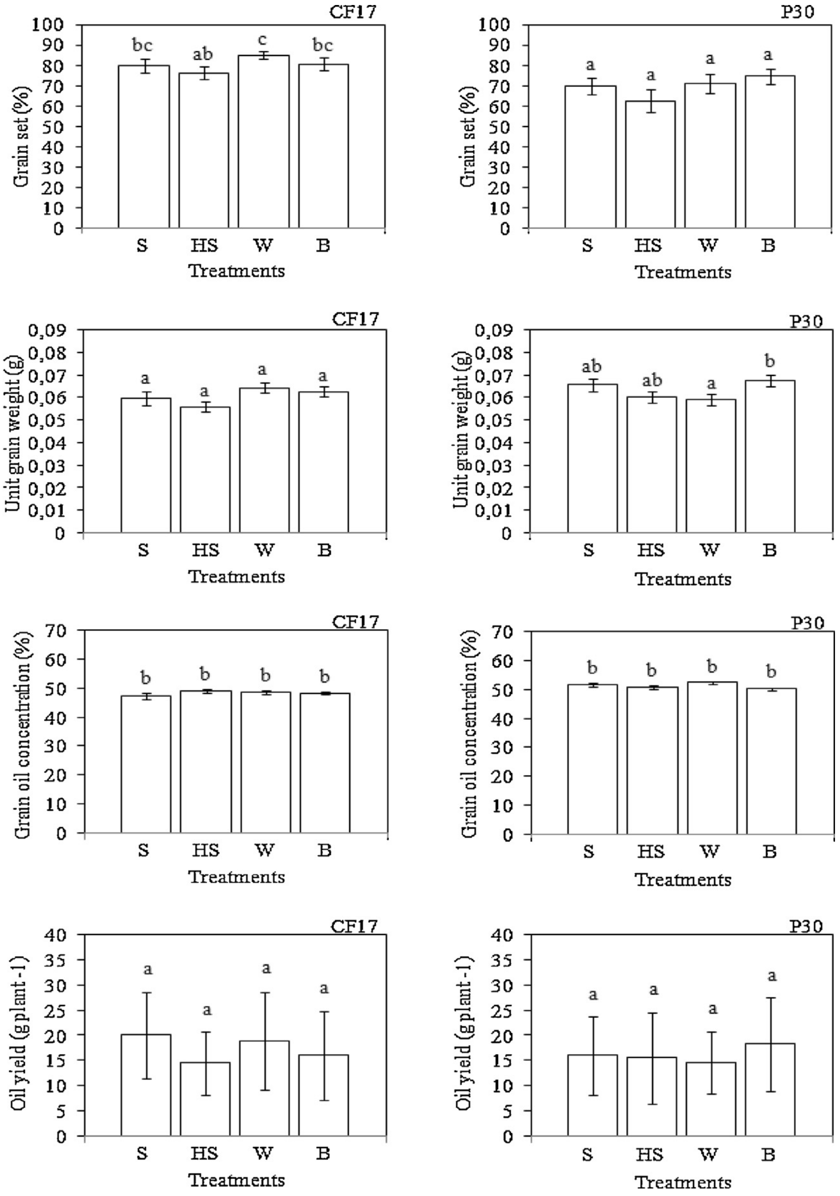

Fig. 2. Whole-head values (means ± 1 standard error of the mean) of grain set, unit grain weight, grain oil concentration and oil yield for the CF17 and P30 sunflower hybrids under several pollination treatments (S: automatic self-pollination; HS: hand self-pollination; W: within-hybrid hand cross pollination; B: between-hybrid hand cross pollination) in Experiment A. Different letters indicate significant differences ($P < 0.05$).

HS, but the applicator was first ‘charged’ with pollen from another head of the same hybrid, then rubbed on the target head; Between-hybrid hand cross pollination (B): as treatment W, but using a head from a different hybrid as pollen source.

2.3. Experiment B

It was intended to simultaneously test the effects of pollen quality and pollen amount on yield components. The six treatments included: Emasculation control (E): daily, newly opened florets were emasculated and bagged heads were otherwise kept unmanipulated until harvest; Low-amount within-hybrid hand cross pollination (WL): as treatment E, but stigmas of emasculated florets were gently rubbed with a low-dose-charged brush with pollen from another head of the same hybrid; High-amount within-hybrid hand cross pollination (WH): as treatment WL, but the brush was two-dose-charged; Low-amount between-hybrid hand cross pollination (BL): as treatment WL, but using heads from a different hybrid as pollen source; High-amount between-hybrid hand cross pollination (BH): as treatment WH, but using heads from a different hybrid as pollen source; Control of mob efficiency as pollen barrier for wind-transported pollen (C): heads of a male-sterile line were kept bagged and unmanipulated until harvest.

2.4. Experiment C

As ExpB, it was intended to simultaneously test the effects of pollen quality and pollen amount on yield components, but it also tested the mechanical effects of particle deposition on stigmas. The seven treatments included: Automatic self-pollination (S) (as described in ExpA); Emasculation control (E), Low-amount within-hybrid hand cross pollination (WL), High-amount within-hybrid hand cross pollination (WH), Low-amount between-hybrid hand cross pollination (BL) and High-amount between-hybrid hand cross pollination (BH) (all as described in ExpB); Mechanical effect of particle deposition on stigmas (ME): florets were ‘pollinated’ with

Table 4
Summary of effects of treatment (T), head sector (S), and interactions (T × S) on grain set and oil yield (Log-linear analysis, Marginal Maximum Likelihood χ^2 tests) and on unit grain weight and grain oil concentration (ANOVA, *F*-tests) of two sunflower hybrids in Experiment A. Statistically significant values are highlighted in **boldface**.

Hybrid	Variables	Source of variation	DF	χ^2/F	*P*-value
P30	Grain set	T	16	20.192	0.2117
		S	8	**29.222**	**0.0002**
		T × S	32	17.86	0.9793
	Oil yield	T	8	3.37936	0.908338
		S	4	**63.58829**	**0.000001**
		T × S	16	7.5	0.9623
	Unit grain weight	T	4	**3.66**	**0.0073**
		S	2	**54.95**	**0.0001**
		T × S	8	0.24	0.9825
	Grain oil concentration	T	4	**15.04**	**0.0001**
		S	2	**9.98**	**0.0001**
		T × S	8	0.40	0.9207
CF17	Grain set	T	12	**28.617**	**0.004503**
		S	6	**30.67**	**0.000029**
		T × S	24	23.26	0.504
	Oil yield	T	8	7.767	0.4565
		S	4	**84.246**	**0.0001**
		T × S	16	6.66	0.9793
	Unit grain weight	T	4	**7.77**	**0.0001**
		S	2	**8.04**	**0.0005**
		T × S	8	0.23	0.9840
	Grain oil concentration	T	4	**10.82**	**0.0001**
		S	2	**7.51**	**0.0008**
		T × S	8	0.60	0.7736

commercial maize starch in the same way as done for high-amount hand cross pollination treatments.

2.5. Determination of yield

Heads were harvested at physiological maturity and stored at −10 °C for later analysis. Since grain set decrease centripetally in sunflower heads (Seiler, 1997; Santalla et al., 2002; Trápani et al., 2003), heads were divided in three concentric sectors of equal radial extent which were sampled separately. In one-eighth of each sector grain set (%) was calculated as (No. filled grains/[No. unfilled grains + No. filled grains]) × 100. The number of unfilled grains included non-functional florets. Unit grain weight (g) was calculated for a subsample of 50 randomly chosen filled grains of each sector which had been previously dried at 65.5 C for 2–3 days. Grain oil concentration (% dry matter) was determined by nuclear magnetic resonance (NMR) using 6–10 g filled grains samples of each sector and sunflower oil as standard at Advanta Semillas (Venado Tuerto, Argentina) or at INTA's Estación Experimental Agropecuaria Balcarce. Oil yield for each sector was calculated as (grain oil concentration × unit grain weight × No. filled grains in one eighth) × 8. Under some treatments, grain set was very low and the number of filled grains was insufficient for the determination of some variables.

2.6. Statistical analysis

Each experiment and hybrid was analyzed separately. Data were subjected to a factorial ANOVA, in which 'treatment' and 'head sector' were the main effects, and *grain set*, *unit grain weight*, *grain oil concentration* and *oil yield* were the response variables. When significant differences for 'treatment' or 'head sector' were detected, means were separated using Tukey test at the 5% level. In cases of non-normality and when origin variables transformation did not work, the variables were analyzed through log-linear analysis (Agresti, 1996) and means were separated with a multiple-comparison non-parametric test (Conover, 1999). For the log-linear analysis data were categorized in classes as follows: (a) in ExpA for grain set in five classes [very low (grain set ≤ 13), low (13 < grain set ≤ 40), medium (40 < grain set ≤ 63), high (63 < grain set ≤ 88) and very high (grain set > 88)], and for oil yield in three classes [low (oil yield ≤ 11.05), medium (11.06 < oil yield ≤ 30.92) and high (oil yield > 30.92)] and (b) in ExpB for grain set in three classes [low (grain set ≤ 25), medium (25 < grain set ≤ 75) and high (grain set > 75)]. So, we included three factors in the log-linear equation.

3. Results

3.1. Experiment A

Within-hybrid hand cross pollination significantly increased grain set (as compared to hand self-pollination) in CF 17. In the other hybrid (P30) no between-treatment differences were found. Unit grain weight was higher under between-hybrid than within-hybrid hand cross pollination in P30. Grain oil concentration and oil yield remained unaffected by the pollination treatments (Fig. 2).

In both hybrids all yield components increased from the central sector to the periphery of the head, except for grain oil concentration, which showed the reverse trend. Values for the central and peripheral sectors always differed significantly, while the behavior of the middle sector varied (values not shown). No sector × treatment interaction was detected for any variable (Table 4).

3.2. Experiment C

Irrespective of pollen dose level, within-hybrid and between-hybrid cross pollination significantly increased grain set, as compared to emasculation control, automatic self-pollination and mechanical-rubbing effect treatments in the hybrid DK4050. High-amount between-hybrid cross pollination significantly increased grain set as compared to low-amount within-hybrid. Automatic self-pollination generated grain set values significantly higher than emasculation control and mechanical-rubbing effect treatments did. Low-amount within-hybrid cross pollination strongly increased the unit grain weight with respect to either dose of between-hybrid cross pollination and to high-amount within-hybrid cross pollination.

Under emasculation control, mechanical-rubbing effect and automatic self-pollination treatments, unit grain weight was higher than in the within- and between-hybrid cross pollination (irrespective of dose level). Within- and between-hybrid cross pollination (irrespective of dose level) very significantly increased grain oil concentration and oil yield as compared to emasculation control and mechanical-rubbing effect treatments. Mechanical-rubbing effect and automatic self-pollination strongly significantly increased grain oil concentration and oil yield with respect to the emasculation control. Between-hybrid (irrespective of dose level) and high-amount within-hybrid cross pollination increased grain oil concentration as compared to the automatic self-pollination treatment. High-amount within-hybrid and low-amount between-hybrid cross pollination increased oil yield as compared to automatic self-pollination (Fig. 3).

Grain set was higher in the periphery than in the middle head sector. Unit grain weight and oil yield increased strongly significantly from the central sector to the periphery of the head. Grain oil concentration showed the reverse trend (values not shown). The interaction sector × treatment was marginally significant for the variable grain set (Table 5).

3.3. Experiment B

Emasculation control and control of mob efficiency produced extremely low values of grain set (4.38% and 3.36%, respectively). Irrespective of pollen dose level, within-hybrid and between-hybrid cross pollination significantly increased grain set as compared to emasculation control and control of mob efficiency. In the hybrid (but not in the male-sterile line) low amount between-hybrid cross pollination produced significantly higher unit grain weight than low amount within-hybrid cross pollination. Grain oil concentration and oil yield remained unaffected by the pollination treatments both in DK4050 and in the male-sterile line (Fig. 4).

In both materials all yield components increased from the central sector to the periphery of the head, except for grain oil concentration, which showed the reverse trend (significantly so in DK4050 only). Values for the central and peripheral sectors always differed significantly, while the behavior of the middle sector varied (values not shown). No sector × treatment interaction was detected for any variable (Table 6).

4. Discussion

4.1. Grain set

In unmanipulated florets (i.e. non-emasculated) of hybrid CF17 grain set increased 8.8% after the addition of same-hybrid pollen in comparison with the manual redistribution of the pollen available in the head, but the addition of pollen of a different hybrid (P30) did not elicited a statistically similar response, which suggests that sensitivity to pollen identity (i.e. pollen quality) was involved in the additional effect. However, when the hybrid P30 was used as ovule parent, grain set was not significantly affected by either pollen quantity or pollen quality. Pollen quality is known to affect reproductive performance in other crops. In coffee, hand cross pollination led to the highest fruit set in comparison to self pollination (Klein et al., 2003).

In the absence of manual pollination, emasculated florets (Experiment B and Experiment C) and florets of a male-sterile line (Experiment B) produced similar and extremely low values of grain set (8% on average), providing a reliable baseline to assess the effects of same- or different-hybrid cross-pollination at different amounts, and at the same time indicating that emasculation did not harm female function. Although stigmas from emasculated florets received a relatively high pollen load (198 pollen grains on average) this amount was insufficient to trigger seed development in most florets (even when in the sunflower ovary there is a single ovule). Stigmatic pollen loads in open-pollinated sunflower amount to ca. 900 pollen grains (Torretta, 2007), contrasting with much lower values in other crops (fewer than 10 pollen grains in buckwheat,

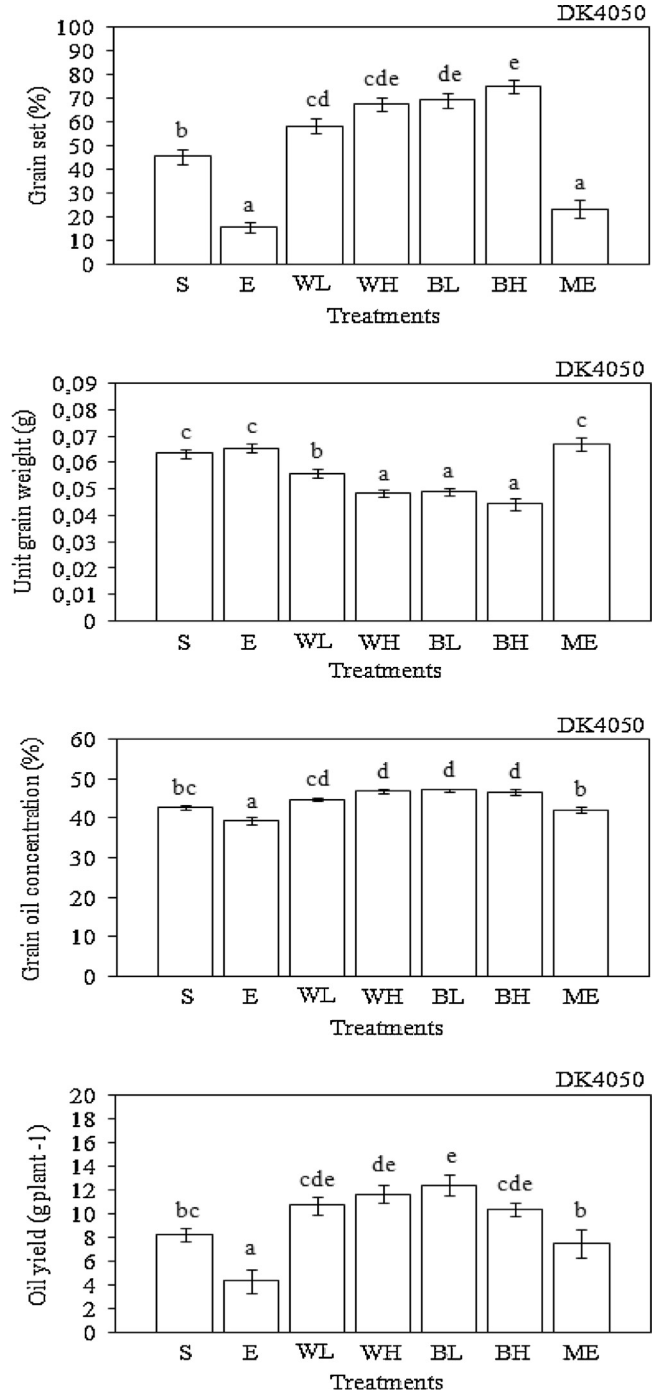

Fig. 3. Whole-head values (means ± 1 standard error of the mean) of grain set, unit grain weight, grain oil concentration and oil yield for the DK4050 sunflower hybrid under several pollination treatments (S: automatic self-pollination; E: emasculation control; WL: low-amount within-hybrid hand cross pollination; WH: high-amount within-hybrid hand cross pollination; BL: low-amount between-hybrid hand cross pollination; BH: high-amount between-hybrid hand cross pollination; ME: mechanical effect of particle deposition on stigmas) in Experiment C. Different letters indicate significant differences ($P < 0.05$).

Table 5
Summary of effects of treatment (T), head sector (S), and interactions (T × S) on grain set, unit grain weight, grain oil concentration and oil yield (ANOVA, *F*-tests) of a sunflower hybrid under several pollination treatments in Experiment C. Statistically significant values are highlighted in **boldface**.

Hybrid	Variables	Source of variation	DF	*F*	*P*-value
DK4050	Grain set	T	7	**52.99**	**0.000001**
		S	2	**4.04**	**0.018500**
		T × S	14	1.88	0.028400
	Unit grain weight	T	7	**30.97**	**0.000001**
		S	2	**55.86**	**0.000001**
		T × S	14	0.46	0.950400
	Grain oil concentration	T	7	**15.23**	**0.000001**
		S	2	**24.02**	**0.000001**
		T × S	14	0.70	0.770500
	Oil yield	T	7	**10.15**	**0.000001**
		S	2	**34.83**	**0.000001**
		T × S	14	0.97	0.489000

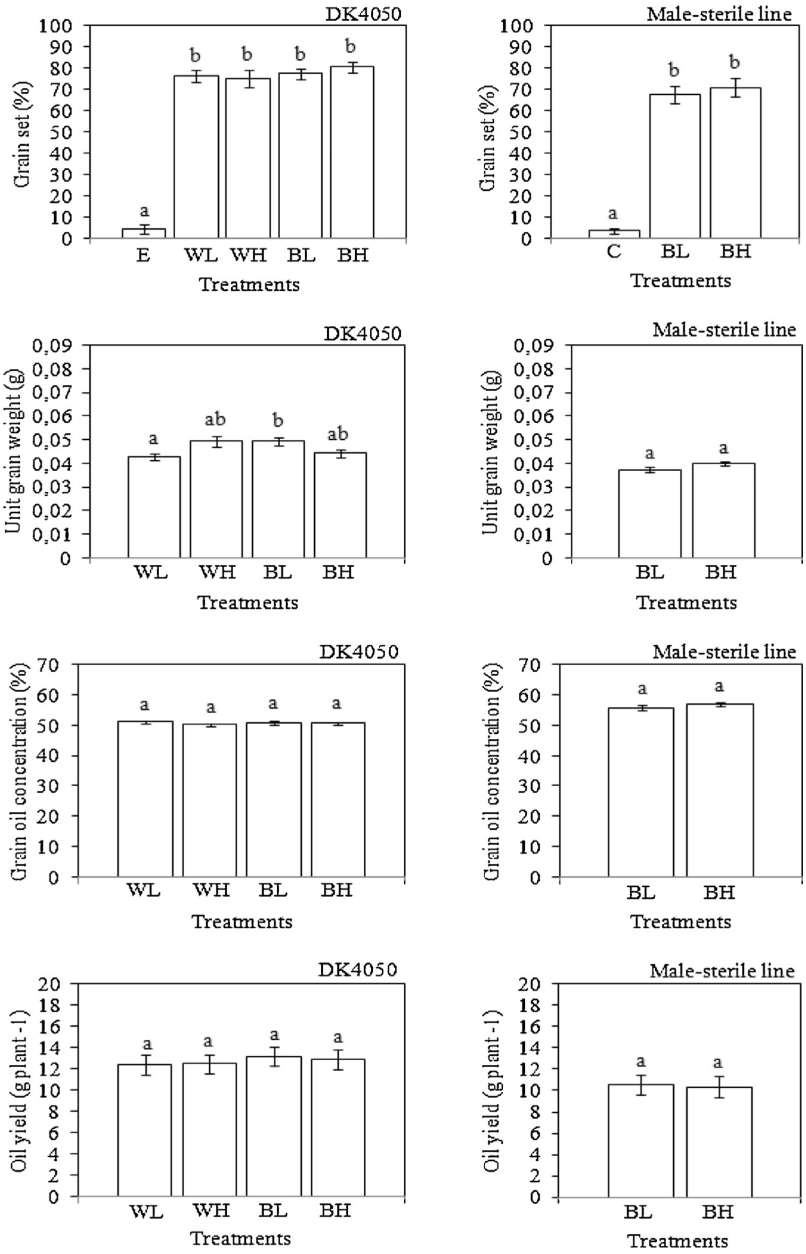

Fig. 4. Whole-head values (means ± 1 standard error of the mean) of grain set, unit grain weight, grain oil concentration and oil yield for the DK4050 sunflower hybrid and a male-sterile line under several pollination treatments (for treatment codes see Fig. 3) in Experiment B. Different letters indicate significant differences ($P < 0.05$).

Table 6
Summary of effects of treatment (T), head sector (S), and interactions (T × S) on grain set (log-linear analysis, marginal maximum likelihood χ^2 tests) and on unit grain weight, grain oil concentration and oil yield (ANOVA, *F*-tests) in a sunflower hybrid and a male-sterile line in Experiment B. Statistically significant values are highlighted in **boldface.**

Hybrid	Variables	Source of variation	DF	χ^2/F	*P*-Value
DK4050	Grain set	T	8	**93.971**	**0.0001**
		S	4	**39.572**	**0.0001**
		T × S	16	13.83	0.6112
	Unit grain weight	T	3	**3.74**	**0.01380**
		S	2	**11.60**	**0.00001**
		T × S	6	0.20	0.97480
	Grain oil concentration	T	3	1.02	0.38870
		S	2	**8.59**	**0.00040**
		T × S	6	0.72	0.63430
	Oil yield	T	3	0.67	0.57550
		S	2	**91.19**	**0.00001**
		T × S	6	0.09	0.99730
Male-sterile line	Grain set	T	4	**52.787**	**0.0001**
		S	4	**23.520**	**0.0001**
		T × S	8	5.582	0.6940
	Unit grain weight	T	1	3.39	0.0719
		S	2	**8.29**	**0.0008**
		T × S	2	0.14	0.8644
	Grain oil concentration	T	1	1.49	0.2300
		S	2	0.91	0.4114
		T × S	2	0.02	0.9844
	Oil yield	T	1	0.11	0.7384
		S	2	**12.13**	**0.0001**
		T × S	2	0.01	0.9996

Björkman, 1995; in maize about 2 pollen grains per exposed silk are sufficient to produce 95% kernel set, Uribelarrea et al., 2002).

In Experiment C grain set in the hybrid DK4050 responded positively to the increase of the pollen amount delivered to stigmas and to the application of different-hybrid instead of same-hybrid pollen. When the extremes were compared (low same-hybrid and high different-hybrid) the difference (16%) reached statistical significance. The results of this experiment support and reinforce the notion, derived from Experiment B, that the hybrid DK4050 is sensitive to the addition of pollen, particularly of pollen from a different hybrid. In highbush blueberry a similar response was found: high pollen loads of different-cultivar increased fruit set in comparison to low pollen loads of same-cultivar (Dogterom et al., 2000). Also in buckwheat seed production increased when pollen loads were increased from 10 to 30 pollen grains per stigma (Björkman, 1995). The lower number of replications used in Experiment B may explain why DK4050 failed to show a differential response in grain set to different amounts of its own or another hybrid's pollen in that experiment.

The inclusion of the automatic self-pollination treatment in Experiment C showed that in DK4050 automatic pollen self-delivery was insufficient for maximizing grain set, which (as discussed above) only were attained when stigmas received supplementary (and especially different-hybrid) pollen. In the hybrids CF17 and P30 no such differences were observed, probably due (a) to the higher self-compatibility level of these hybrids and (b) to the fact that an excess of self pollen was available because in Experiment A florets were not emasculated.

4.2. Unit grain weight

We detected an effect of foreign pollen in unit grain weight. P30 unit grain weight increased 17% following the addition of CF17 pollen as compared to the addition of P30 pollen. This increase seems not be due to a compensation between number of grains and individual grain weight, because also grain set tended to be higher in P30 heads supplemented with CF17 pollen. Likewise, in DK4050 unit grain weight was 14% higher with the addition of P30 pollen in low-amount than with the addition of its own pollen under low-amount too (experiment B), suggesting a beneficial effect of different-hybrid pollen on this yield component. However, the opposite result was observed in the same hybrid in a different year (experiment C). Regardless of the direction of this effect, the sensibility to pollen quality was only expressed at low-amount conditions, and disappeared with higher pollen amounts, suggesting that there is a threshold pollen quantity per stigma over which yield is no longer affected by pollen quality. Thus, unit grain weight showed a combined influence of pollen quality and pollen amount.

The Experiment A indicates that some sensitivity to pollen quality and/or amount (showed by CF17 as regards grain set) and pollen quality (showed by P30 as regards unit grain weight) exists in these hybrids. Note that pollen of CF17 exerted a consistently positive effect on P30 (reaching statistical significance on unit grain weight), while pollen of P30 consistently depressed the performance of CF17 (admittedly, without reaching statistical significance). A subtle asymmetric compatibility effect between these hybrids may be involved.

4.3. Grain oil concentration and oil yield

In experiment A florets were not emasculated, which may explain why CF17 and P30 failed to show a differential response in grain oil concentration and oil yield to different amounts of its own or another hybrid's pollen. In non emasculated florets the presence of their own pollen would weaken the effect of pollen addition irrespective of quality or amount.

In Experiment C grain oil concentration and oil yield per plant in DK4050 showed an effect of pollen type and amount, since florets receiving either high amounts of same-hybrid pollen or low amounts of different-hybrid pollen produced significantly higher values of both variables than emasculated florets did. A similar effect (although of lower magnitude) was obtained following the application of maize starch to emasculated florets, probably due to the redistribution across the head of the residual pollen left after emasculation, which could enhance the arrival of pollen to stigmas in some way. Note that the 'redistribution effect' is present in all manual pollination treatments as a sort of baseline upon which the effects of pollen addition are superimposed. Additional research is needed to separate redistribution and addition effects.

5. Conclusion

Our results (a) demonstrate for the first time that the pollen amount and pollen quality that arrive to the stigma have a joint effect on sunflower grain set, (b) suggest that some yield crop components could be sensitive to this joint effect, and (c) remark the probable positive effect of the pollen redistribution across the head. Overall, results underline the importance of pollinators for the crop, and point out to possible mechanisms that explain their beneficial effect.

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