

Full Length Research Paper

Effects of honeybee (*Apis mellifera*) pollination on seed set in hybrid sunflower (*Helianthus annuus* L.)

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This study was carried out to determine the efficiency of pollination with honeybee (*Apis mellifera*) on sunflower hybrid seed production under different types of pollination during 2005 and 2006 in Mustafakemalpaşa-Bursa, Turkey. Three pollination types (1) in cages with honeybees, (2) hand pollination (in cages) and (3) in cages without honeybees were used in crosses between two male and three female parents in all possible combinations. The experiment factors; pollination types, male parents and female parents were designed in a split-split plot of randomized complete block. Seed set ratios were 98-99% for pollinations in cages with honeybees or by hand as artificial whereas this ratios was reduced to a level of 4-5% by pollination in cages without honeybees in the both experimental years. Pollination in cages without honeybees produced 93-94% less number of filled seeds per head compared with pollinations by hand and in cages with honeybees. In addition, pollinations in cages with honeybees and by hand resulted in higher 100 seed weight compared with pollination in cages without honeybees. Pollination in cages with honeybees and by hand increased seed yield per head by about 206 and 226%, respectively, compared with pollination in cages without honeybees. Results indicated that the use of honeybees for sunflower hybrid seed production improved seed set ratio, 100 seed weight, number of filled seed per head and seed yield per head.

Key words: Sunflower (*Helianthus annuus* L.), honeybees, pollination, seed set, hybrid seed yield, cages.

INTRODUCTION

The importance of honeybees (*Apis mellifera*) in the production of sunflower (*Helianthus annuus* L.) is high (Nye and Mackenson, 1968 and 1970; Tanda, 1984; Olmstead and Wooten, 1987; Henning et al., 1992; Singh and Singh, 1992; Gordon et al., 1995; Degrandi-Hoffman and Buchmann, 1995). Individual sunflower florets are rarely self-pollinated and need pollen transferred to them from other florets, which can be done by using honeybees (Putt, 1940; Furgala, 1954; Free, 1963). Seed yields have been increased by taking honeybee colonies to sunflower crops (Ponomareva, 1958; Langridge and Goodman, 1981; Gordon et al., 1995). Bees certainly are essential in

seed production for male sterile (i.e. non pollen producing) sunflowers because pollen must be transferred from male-fertile to male sterile plants (Degrandi-Hoffman and Chambers, 2006). Hybrid seed in sunflower is produced by using cytoplasmic male sterile (CMS) lines as female parents. The CMS line (A line) is pollinated with maintainer male fertile line (B line) for its maintenance and with restorer fertile line (R line) for hybrid seed production.

Sunflower pollen is heavy and sticky so cannot be carried by wind (Yadav et al., 2002). Therefore, pollen is transferred from male lines to female lines by insects. Earlier studies suggested that more seed is set when honey bees forage on sunflowers (Parker, 1981; Paiva et al., 2002). However, Degrandi-Hoffman and Chambers (2006) reported that in self-fertile cultivars such as hybrid sunflowers, self pollination can produce seed set by bees

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Table 1. Mean air temperature and total monthly precipitation in 2005, 2006 and 1990-2007 averages at Mustafakemalpaşa.

Month	Temperature (°C)			Precipitation (mm)		
	2005	2006	1990-2007	2005	2006	1990-2007
March	9	10	8	78	57	67
April	14	14	13	43	13	75
May	18	19	18	36	9	41
June	22	22	22	21	63	34
July	25	24	25	55	2	18
August	25	27	25	3	3	25
Total	-	-	-	236	147	260

moving move among florets collecting nectar and pollen. To grow hybrid sunflower seed, male-fertile (R line) cultivars (pollen-producing, hereafter 'males') and malesterile (A line) cultivars (only nectar producing, hereafter 'females') are planted in separate rows within a field.

Sunflower offers a scope of developing new and superior varieties through heterosis breeding (Sing et al., 1984). High heterosis for yield characters in sunflower has been widely reported. Gundaev (1970) showed that hybrids in USSR yielded as much as 50% more than existing cultivars. Fick and Zimmer (1976) in the USA reported that hybrids yielded up to twice as much as check cultivars. Considerable heterotic effects were reported for head diameter, seed weight, plant height and seed yield (Putt, 1966; Chaudhary and Anand, 1984; Kadkol et al., 1984; Singh et al., 1984).

In Turkey, sunflower is grown on around 0.5 M ha per year with average seed yields of 1.20-1.40 t ha⁻¹ (FAO-STAT, 2005), with the largest sunflower production center in the Marmara Region. In the last 20 years, seed production of sunflower in Turkey has greatly increased in areas where high yielding and adapted hybrid cultivars have been planted. In Turkey, during the same period, the use of hybrid cultivars has reached 95% of plantings. The importance of hybrid breeding in sunflower has increased recently because of their higher seed yield than cross-pollinated varieties for sunflower oil production. Hybrid sunflowers are more stable, highly self-fertile and more uniform at maturity (Dedio and Enns, 1976; Seetharam, 1979). Honeybee pollination is therefore essential for production of sunflower hybrid seed. In many earlier studies, it was reported that seed yield and seed set were greatly increased by visit of honeybees (Ponomareva, 1958; Free, 1963; Moreti et al., 1996; Tan et al., 2002).

The aim of this study was to determine the pollination efficiency using honeybees on seed set, number of filled seed per head, seed weight and seed yield per head in sunflower hybrid seed production under different types of pollination in the Marmara Region of Turkey.

MATERIALS AND METHODS

Field studies were conducted in 2005 and 2006 at the experimental field of Mustafakemalpaşa Vocational School, Uludağ University,

Mustafakemalpaşa, Bursa, Turkey (40°02'M, 28°24' E and altitude 25 m above sea level) on a clay soil having 0.1% total nitrogen content, 0.41 kg ha⁻¹ phosphorus, 7.70 kg ha⁻¹ exchangeable potassium and 3.0% organic matter.

The local climate is temperate, summers are hot and dry, winters are mild and rainy. Average annual rainfall is 703 mm and mean monthly temperature is 14.6°C. Total monthly precipitation and mean air temperature data during the sunflower growing period are presented in Table 1. Total rainfall from March to August was 236 and 147 mm in 2005 and 2006, respectively. This corresponded to 33.5 and 21%, respectively, of the annual precipitation. Mean air temperature during the flowering of sunflower was approximately 14.6°C in the both experimental years. Mean air temperature during period flowering of the plants was 21-22°C.

Weather during the second year of the study was very dry with rainfall 113 mm below average. In 2005, precipitation during the growing period of sunflower was 24 mm below normal but with favorable distribution.

Three cytoplasmic male sterile lines; 'CMS 01', 'CMS 10' and 'CMS 23' and fertility restorer lines; viz 'RHA 03' and 'RHA 10' used as the parent in the study were improved from certain germplasm sources by the Uludağ University, Bursa, Turkey.

The experimental factors; pollination types, male parents and female parents were in a split-split plot arrangement of randomized complete block design with three replications. Main plots were three pollination types; in cages with bees, hand pollination (in cages) and in cages without bees. Split plots were male parents (restorers); RHA 03 and RHA 10. Split-split plots were three female parents CMS 01, CMS 10 and CMS 23. In the experiment, two cages involving 3 different male lines were used with one restorer line and for each pollination type. Eighteen cages were established in the experiment because of containing three pollination types and three blocks. Different three female lines (CMS lines) and one male line (restorer line) were sown in planting ratio of 1 restorer : 3 CMS (as two rows for each CMS line) in the each male cage. Row spacing was 60 cm while plant to plant distance, 30 cm. Each 4 x 5.4 x 2.5 m cage had an area of 21.6 m² (5.4 x 4.0 m). To prevent bees from escaping and to impede the entrance of insects from out, the cages were covered by 2.5 m plastic material with 2 mm holes. Male and female lines were crossed by hand honeybee pollination and no pollination by hand or bees at all possible combinations and six experimental hybrids were obtained for each pollination type in the both years. All the measurements were made from female head hybrid seeds.

A small bee hive was used in the each cage with bee. Heads of both female and male plants were bagged in plots where pollination was by hand, to prevent possible wind-pollination.

Plantings were done on 18 April 2005 and 21 April 2006 in both years. Sixty kilograms of nitrogen per hectare as diammonium phosphate (DAP, 18-46-0) composed fertilizer was applied prior to sowing and a further 60 kg N ha⁻¹ was added when the plants were

Table 2. Analysis of variance and means in terms of certain seed characters for three different pollination types, two male parents, and three female parents in sunflower hybrid seed production during 2005 and 2006.

Treatment	Seed set ratio (%)		Number of filled seeds per head		100 seed weight (g)		Seed yield per head (g)	
	2005	2006	2005	2006	2005	2006	2005	2006
Pollination types								
In cages with bees	98.7 a	98.5 a	1172 a	1100 a	5.47 a	6.69 a	64.8 a	70.4 a
Hand pollination	98.8 a	97.9 a	1106 a	924 b	4.83 b	7.09 a	63.2 a	63.5 b
In cages without bees	4.7 b	4.6 b	74 b	62 c	1.62 c	1.68 b	19.4 b	22.2 c
LSD (0.05)	1.83	0.64	89	92	0.26	0.58	4.2	2.8
Restorer (male)								
RHA 03	66.9	67.3	729 b	684	3.91	5.55 a	51.6	55.3 a
RHA 10	67.9	66.8	839 a	707	4.04	4.76 b	46.7	48.8 b
LSD (0.05)	ns	ns	87	ns	ns	0.15	ns	3.9
Cytoplasmic male sterile (female)								
CMS 01	67.0	67.8	887 a	799 a	4.28 a	4.85 b	56.1 a	54.7 a
CMS 10	68.2	66.5	861 a	711 b	3.98 ab	4.77 b	54.0 a	51.2 ab
CMS 23	67.0	66.8	604 b	577 c	3.66 a	5.85 a	37.3 b	50.2 b
LSD (0.05)	ns	ns	51	37	0.34	0.42	5.6	3.6
Analysis of variance								
Source	MS	MS	MS	MS	MS	MS	MS	MS
Block	ns	ns	ns	ns	ns	ns	ns	ns
Pollination type (A)	**	**	**	**	**	**	**	**
Male (B)	ns	ns	*	ns	ns	**	ns	*
Female (C)	ns	ns	**	**	**	**	**	*
Male x Female (F ₁)	ns	ns	ns	**	ns	ns	ns	ns
AxB	ns	ns	ns	ns	ns	**	ns	ns
AxC	ns	ns	**	**	ns	**	ns	*
AxBxC	ns	ns	ns	**	ns	*	ns	**

30-40 cm in height. After planting, Linuron was sprayed at a rate of 20 cm³ m⁻² for weed control. Hand hoeing for weeds were done when necessary. Previous crop of the field trial was soybean in the both years. Irrigation was applied to reach field capacity level of the soil at three critical growth periods of sunflower: heading, flowering and milking. Prior to flowering of the plants, small bee hives were placed in the designated cages.

RESULTS AND DISCUSSION

There were significant differences for all plant characteristics investigated among pollination types. Differences between male parents (restorer lines) were significant ($p \leq 0.01$) for number of filled seeds per head in 2005; for 100 seed weight and seed yield per head in 2006. Female parents (CMS lines) were significantly different for all characteristics observed in the both experimental years except seed set. On the other hand, male x female interaction was only significant ($p \leq 0.01$) for number of filled seeds per head in 2006, while pollination type x female and pollination type x male x female interactions were found significantly for number of filled seeds per head, 100 seed weight and seed yield per head in 2006. Also, pollination type x female interaction was statistically signi-

ficant for number of filled seeds per head in 2005 (Table 2). Pollination in cages with bees and pollination by hand produced higher seed set ratio compared with pollination in cages without bees in the both years. Seed set ratios were 98-99% for pollinations with honeybees or by hand whereas this ratio was reduced 4-5% by pollination in cages without honeybees. The number of filled seeds per head ranged from 924-1172 seeds/head for pollinations by hand or in cages with honeybees to 62-74 seeds/head for pollination in cages without honeybees. When compared as a percentage, the number of filled seeds per head realized was 93-94% less in cages without honeybees.

In our study, seed weight showed significant differences according to the years. In 2005, pollination in cages with honeybees gave higher 100 seed weight (5.47 g) compared with pollination by hand (4.83 g) and pollination in cages without honeybees (1.62 g). Whereas, in 2006, pollinations in cages with honeybees and by hand resulted in significantly higher 100 seed yield than pollination in cages without honeybees. There was no a significant difference between pollinations in cages with honey bees and by hand in seed weight. Pollination in cages with honeybees and by hand produced, on average 198 -

322% more seed weight than pollination in cages without honeybees. Responses of pollination types were different across the experiment years. In 2005, pollination in cages with honeybees and by hand produced higher seed yield per head compared with pollination in cages without honeybees while 2006, pollination in cages with honeybees gave significantly higher seed yield than the other pollination types. Pollinations in cages with honeybees and by hand increased seed yield per head by about 206 and 226%, respectively, compared with pollination in cages without honeybees.

As a results, pollination in cages with honeybees and pollination by hand increased significantly percentage of seed setting, number of filled seeds per head, 100 seed weight and seed yield per head, compared with pollination in cages without honeybees. In a similar study, Moreti et al. (1996) reported that the number and weight of seeds and percentage of seed setting were significantly higher in the visited plant by insect (579.3, 41.2 g and 82.4% and 457.0, 23.4 g, 79.7% in 2 trials) than in plants protected by cages where insects were excluded (81.5, 2.2 g and 1.2% in first trial and 111.9, 3.2 g and 28.5% in the second). In another study, Kumar and Singh (2003) found that the number of filled seeds per capitulum (728.2), seed set (75.5%) and 1000 seed weight (55.9 g) was highest with hand + insect pollination, than open-to-all insects. Also, Calmasur and Ozbek (1999) stated that the highest 1000 seed weight, number and weight of filled seeds per head (52.1 g, 1150.7 and 67.2 g) were obtained under natural conditions, while the lowest 1000 seed weight, number and weight of filled seeds (19.9 g, 373.3 and 16.4 g) were obtained from plants in cages with no bees. The same authors added that seed set rates were 86.8% under natural conditions, 67.8% in cages with 2 beehives and 31.5% in cages with no bees. Similar results were obtained in many earlier experiments (Panda et al., 1993; Arya et al., 1994; Vaisampayan and Sinha, 2000; Tan et al., 2002). Our findings of increased pollination using honeybees in sunflower crops correspond to those of previous works.

There were no significant differences among male lines (restorer lines) in seed setting ratio for the both experimental years. On average, seed set ratio was 67.0% for male lines. On the other hand, male lines responded differently in number of filled seeds per head over 2 years. In 2005, RHA10 produced higher number of filled seeds per head than on the RHA03, whereas differences among male lines were not significant for number of filled seeds per head in 2006 (Table 2). Seed yield per head and 100 seed weight of RHA03 were higher than those of RHA 10 in 2006 while male lines were not different for the two yield components in 2005. As seen in Table 2, 100 seed weight and seed yield per head ranged between 3.9-5.5 and 51.6-55.3 g for RHA 03 and between 4.0- 4.8 g and 46.7- 48.8 g for RHA10, respectively. Stamm and Shuster (1989) detected that in the presence of bees, seed set was 70-80% in male – fertile sunflower plants. In a similar

study, Rao et al. (1995) reported that seed set was higher on insect-pollinated male fertile plants (76.4%) than on hand-pollinated male-fertile plants (16.9%).

Differences among female parents were not statistically significant in only seed set ratio for two experimental years. Seed set ratio ranged between 66.5 and 68.2% according to female parents (Table 2). Female parents responded differently in number of filled seeds per head, 100 seed weight and seed yield per head in the each years. Female parents, CMS01 and CMS10 gave higher number of filled seeds per head compared with CMS23 in 2005, while number of filled seeds per head of CMS01 was significantly higher than that of the other female lines in 2006. Also, In 2005 CMS01 resulted in higher 100 seed weight than CMS23 while CMS10 was in the middle in term of this character. Whereas, in 2006, produced higher 100 seed weight compared with CMS01 and CMS10, in 2005, CMS01 and CMS10 increased seed yield per head by about 50.4 and 44.8%, respectively. In 2006, seed yield per head of CMS01 was higher than that of CMS23 and CMS10 was in the middle for this character. In similar study, Rajagopal et al. (1999) reported that in the case of the female parent, the filled seed weight, seed filling percentage and seed oil content were highest in the plot pollinated only by honeybees. Several previous studies revealed that in the presence of bees, seed set was 70-80% in male sterile plants (Stamm and Shuster, 1989; Rao et al., 1995).

The pollination type x female interaction was statistically significant in the both experimental years. A significant pollination type x female parent interaction effect revealed that among female lines, CMS 01 and CMS 10 had higher number of filled seeds per head than CMS23 from pollination in cages with honeybees and pollination by hand whereas there were no differences between female lines in terms of this character from pollination in cages without honeybees however, their mean values were lower (Figures 1 and 2). Male x female interaction effect on number of filled seeds per head was statistically significant in 2006, however, this interaction was variable over the years. On the other hand, significant male x female interaction effect indicated that differences between the experimental hybrids were significant in number of filled seed per head over all the pollination types in 2006. Hybrids, CMS01 x RHA03 and CMS10 x RHA03 were not differences in number of filled seeds per head, while differences between CMS01 x RHA10 and CMS10 x RHA10 in this character were statistically significant (Figure 3). On 100 seed weight, both the pollination type x male parent and pollination type x female parent interaction effects were significant in only 2006. These two interactions exhibited that either male parents or female parents were significantly different in 100 seed weight at pollination in cages with honeybees and pollination by hand while there were no differences between these parents from pollination in cages without honeybees (Figures 4 and 5).

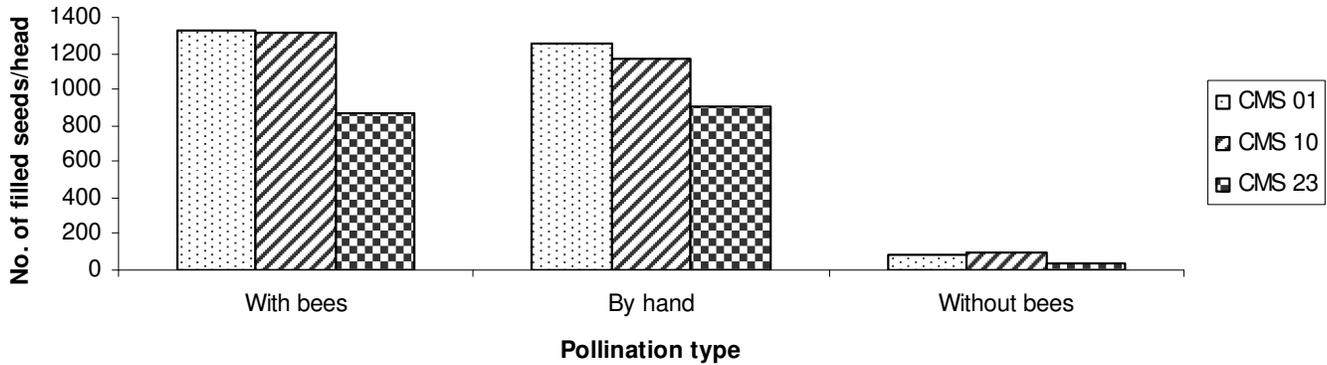


Figure 1. Number of filled seeds per head for female parents at different pollination types in 2005.

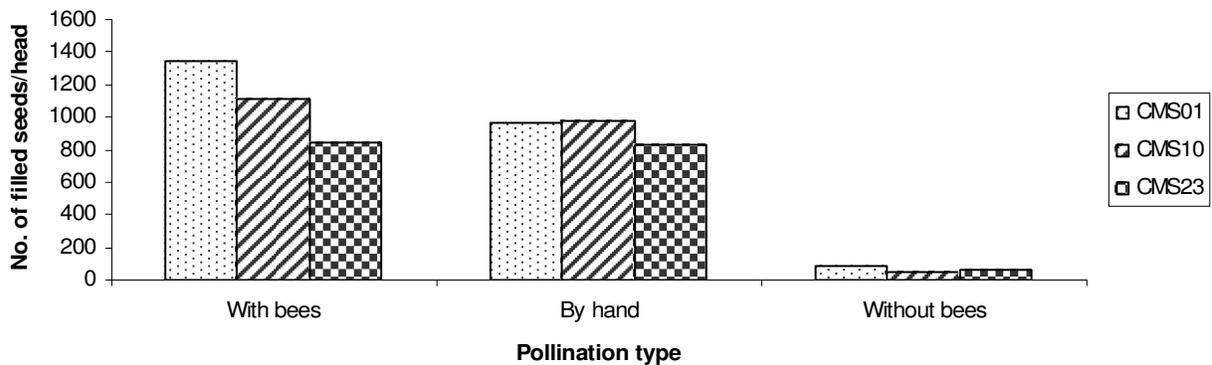


Figure 2. Number of filled seeds per head for female parents at different pollination types in 2006.

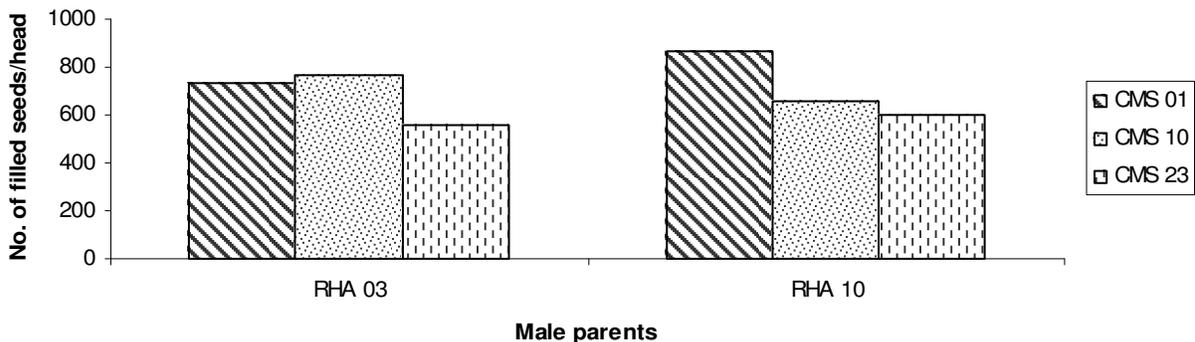


Figure 3. Number of filled seeds per head for female parents in the each male parent in 2006.

Significant pollination type x female parent interaction for seed yield per head showed that there were no differences among female parents either from pollination in cages with honeybees or from pollination in cages without honeybees however female parents responded differently at pollination by hand pollination (Figure 6). A previous study by Rao et al. (1995) showed that seed set was higher on insect-pollinated plants (MS 78.8%, MF 76.4%) than on hand-pollinated plants (MS 15.4%, MF 16.9%), and seed yields were about 3 times as high.

Weather in second year of the study was drier compared with the first year. Although, plants were irrigated at three critical development periods: heading, flowering and milking, treatment effects and their interactions responded differently according to the experimental years. It is likely that differences between the years in terms of treatment effects and their interactions were mainly due to differing responses differently of parent lines to environmental conditions such as soil, temperature and water.

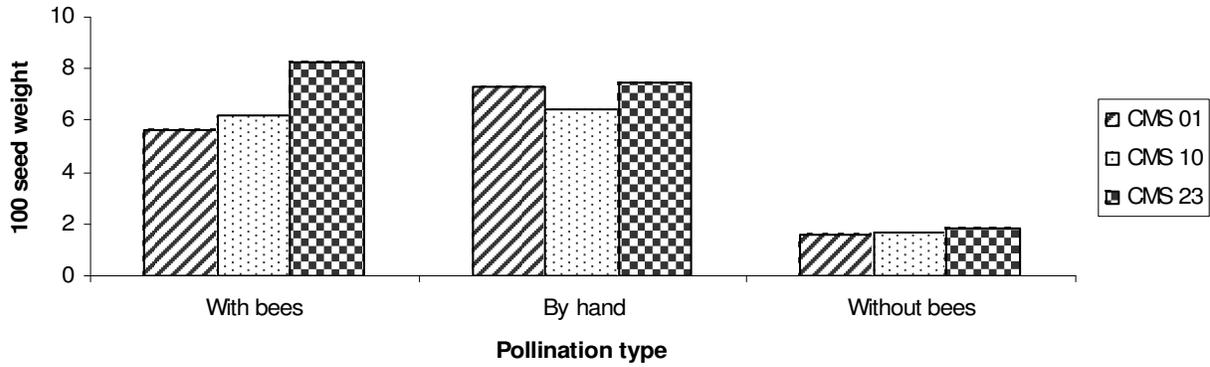


Figure 4. 100 seed weights for female parents at different pollination types in 2006.

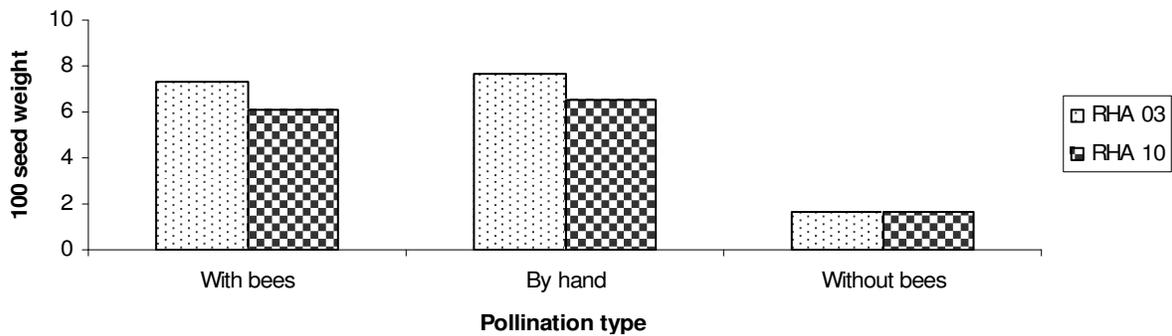


Figure 5. 100 seed weights for male parents at different pollination types in 2006.

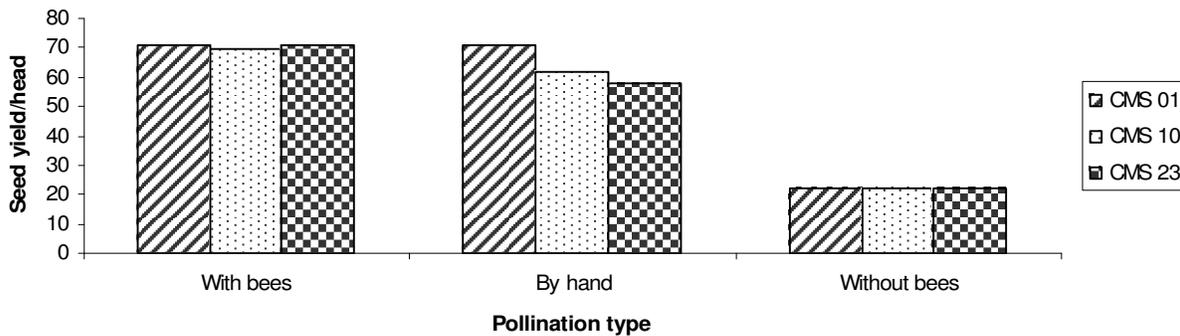


Figure 6. Seed yield per head for female parents at different pollination types in 2006.

Generally, in a drier experimental year (2006) pollination type x female and pollination type x male interaction effects were statistically significant in many characters measured. Significant interactions revealed that the male and female lines could respond differently according to pollination types in drier years and regions and generally where environmental conditions were unfavorable.

Conclusion

Pollination by honeybees is a critical input in sunflower hybrid seed production. Our results, from 2-year period,

indicate that three pollination types; plants caged with honeybees, plant caged without honeybees and plants pollinated by hand, influenced seed set ratio, number of filled seeds per head, seed weight and seed yield per head. Pollination in cages with honeybees and pollination by hand increased seed yield per head by about 206 and 226%, respectively, compared with pollination in cages without honeybees. Pollination in cages with honeybees and pollination by hand as artificial produced higher seed setting ratio compared with pollination in cages without honey-bees. Number of filled seed per head decreased from 924-1172 seeds/head for pollination by hand or in

cages with honeybees to 62-74 seeds/head for pollination in cages without honeybees. Also, pollinations in cages with honeybees and by hand produced 198-322% more 100 seed weight than pollination in cages without honeybees. Both male and female lines responded similarly in number of filled seeds per head. Responses of male lines were different according to the years for the other characters. Female lines, CMS01 and CMS10 gave higher number of filled seeds per head and seed yield per head compared with CMS23. Female lines, also responded differently according to the years in 100 seed weight. Interaction effects in the characters observed were not stable over the years.

REFERENCES

- Arya DR, Sihag RC, Yadav PR (1994). Role of insect pollination in seed yield of sunflower *Helianthus annuus* L. Indian Bee J. 56 (3/4): 179-182.
- Calmasur O, Ozbek H (1999). Pollinator bees (Hymenoptera, Apoidea) on sunflower *Helianthus annuus* L. and their effects on seed setting in the Erzurum Region. Turk. J. Biol. 23(1): 73-79.
- Chaudhary SK, Anand IJ (1984). Heterosis and inbreeding depression in sunflower. Crop Imp. 11: 15-19.
- Dedio W, Enns H (1976). Breeding of early-maturing sunflower hybrids. In: Abstracts of Papers 7th International Sunflower Conference, Krasnodar, USSR. DeGrandi-Hoffman G, Buchmann SL (1995). Some new perspectives on the pollination of hybrid sunflowers. Am. Bee J. 135(9): 628-629.
- DeGrandi-Hoffman G, Chambers M (2006). Effects of honeybee (Hymenoptera: Apidae) foraging on seed set in self-fertile sunflowers *Helianthus annuus* L. Environ. Entomol. 35(4): 1103-1108.
- DeGrandi-Hoffman G, Buchmann SL (1995). Some new perspectives on the pollination of hybrid sunflowers. Am. Bee J. 135(9): 628-629.
- FAO/ST AT, (2005). FAO Statistical Yearbook. Fick GN, Zimmer DE (1976). Yield stability of sunflower hybrids and open-pollinated varieties. In: Abstracts of Papers 7th International Sunflower Conference, Krasnodar, USSR.
- Fick GN, Zimmer DE (1976). Yield stability of sunflower hybrids and open-pollinated varieties. In: Abstracts of Papers 7th International Sunflower Conference, Krasnodar, USSR.
- Free JB (1963). The behaviour of honeybees on sunflower (*Helianthus annuus* L.). J. Appl. Ecol. 1(1): 19-27.
- Furgala B (1954). The effect of the honeybee, *Apis mellifera* L., on the seed set, yield and hybridization of the cultivated sunflower, *Helianthus annuus* L. Entomological Society Manitoba Proceeding 10: 28-29.
- Gordon DM, Barthell JF, Page Jr. RE, Fondrk MK, Thorp RW (1995). Colony performance of selected honeybee (Hymenoptera: Apidae) strains used for alfalfa pollination. J. Econ. Entomol. 88(1): 51-57.
- Gundaev AI (1970). The use of the character of male sterility in interline hybridization of the sunflower. All-Un. Inst. Oil, Essent, Krasnodar, USSR. Reter. Plant Breed. Abstr. 40, 4529.
- Henning JA, Peng YS, Montague MA, Teuber LR (1992). Honeybee (Hymenoptera, Apidae) behavioral-response to primary alfalfa (Rosales, Fabaceae) floral volatiles. J. Econ. Entomol. 85:233-239.
- Kadkol GP, Anand IJ, Sarma RP (1984). Combining ability and heterosis in sunflower. Indian J. Genet. Plant Breed. 44(3): 447-451.
- Kumar M, Singh R (2003). Pollination efficiency of *Apis mellifera* in seed production of sunflower *Helianthus annuus* L. J. Entomol. Res. 27(2): 131-134.
- Langridge DF, Goodman RD (1981). Honeybee pollination of sunflower cultivars Hysun 30 and Sunfola. Aust. J. Exp. Agric. Husb. 21: 435-438.
- Moreti AC, de CC, de Silva RMB, de Silva ECA, Alves MLTMF, Otsuk IP (1996). Increase of sunflower *Helianthus annuus* L.) seed production by pollinating insect action. Sci. Agric. 53 (2/3): 280-284.
- Nye WP, Mackensen O (1968). Selective breeding of honeybees for alfalfa pollen collection. J. Apic. Res. 7: 21-27.
- Nye WP, Mackensen O (1970). Selective breeding of honeybees for alfalfa pollen collection: with tests in high and low alfalfa pollen collection regions. J. Apic. Res. 9 (2): 61-64.
- Olmstead AL, Wooten DB (1987). Bee pollination and productivity growth: the case of alfalfa. Am. J. Agric. Econol. 69: 56-63.
- Paiva GJ de, Terada Y de, Toledo AA (2002). Behavior *Apis mellifera* L. africanized honeybees in sunflower (*Helianthus annuus* L.) and evaluation of *Apis mellifera* L. colony inside covered area of sunflower. Acta Sci. 24 (4): 851-855.
- Panda P, Sontakke BK, Panda B (1993). Effect of different modes of pollination on yield of sunflower and niger. J. Insect Sci. 6(1): 75-77.
- Parker FD (1981). Sunflower pollination. Abundance, diversity and seasonality of bees and their effect of seed yields. J. Apic. Res. 20(1): 49-61.
- Ponomareva EG (1958). Results of mass experiments on the use of bees as pollinators of entomophilic agricultural plants. Biul. Nauthno-Tekh. Inform (Nauchno-Issled. Inst. Pchelovod.) 3-4: 27-28.
- Putt EE (1940). Observations on morphological character and flowering processes in the sunflower (*Helianthus annuus* L.). Sci. Agric. 21: 167-179.
- Putt EE (1966). Heterosis, combining ability and predicted synthetics from a diallel cross in sunflower (*Helianthus annuus* L.). Can. J. Plant Sci. 46: 59-67.
- Rajagopal D, Veeresh GK, Chikkadevaiah, Nagaraja N, Kencharaddi RN (1999). Potentially of honeybees in hybrid seed production of sunflower (*Helianthus annuus* L.). Indian J. Agric. Sci. 68(1): 40-43.
- Rao G, Nadre KR, Suryanarayana MC (1995). Studies on pollination of male sterile line for foundation seed production in hybrid sunflower. Indian Bee J. 57 (4): 170-173.
- Seetharam A (1979). Breeding strategy for developing higher yielding varieties of sunflower. Symposium on Research and Development Strategy for Oilseed Production, New Delhi, India.
- Singh SB, Labana KS, Virk DS (1984). Heterosis in variety x inbred crosses of sunflower. Crop Imp. 11: 35-38.
- Singh RP, Singh PN (1992). Impact of bee pollination on seed yield, carbohydrate composition and lipid composition of mustard seed. J. Apic. Res. 31:128-133.
- Stamm UI, Shuster W (1989). Studies on pollination and fertilization relationship in sunflower (*Helianthus annuus* L.). Angewandte Botanik 63 (5-6): 429-437.
- Tan AS, Ozturk AI, Karaca U (2002). Effect of honeybee pollination on seed yield and quality of sunflower. Anadolu 12(1): 1-26.
- Tanda AS (1984). Bee pollination increases yield of 2 interplanted varieties of Asiatic cotton (*Gossypium arboreum* L.). Am. Bee J. 124: 539-540.
- Vaishampayan S, Sinha SN (2000). Effect of caging on bee behaviour and pollination efficiency of *Apis mellifera* on seed production of hybrid sunflower. Agric. Sci. Digest 20(2): 81-83.
- Yadav RN, Sinha SN, Singhal NC (2002). Honeybee (*Apis spp.*) pollination in sunflower hybrid seed production: Effect of planting design on honeybee movement and its operational area. Apimondia Standing Commission of Pollination and Bee Flora. <http://www.apimondia.org/apiacta/slovenia/en/yadav.pdf>