Indian Journal of Agricultural Sciences **84** (11): 1389–94, November 2014/Article

Foraging behaviour of honeybees (*Apis* spp.) (Hymenoptera: Apidae) in hybrid seed production of Indian mustard (*Brassica juncea*)

ANIRUDDHA MAITY[1], S K CHAKRABARTY[2] and J B YADAV[3]

Indian Agricultural Research Institute, New Delhi 110 012

Received: 13 February 2013; Revised accepted: 30 July 2014

ABSTRACT

Pollination of entomophilous crops by honeybees is considered as one of the effective and inexpensive method for improving the crop yield and its quality. In most of the crops, effective pollination is prerequisite for fruitful fertilisation. Hence, we investigated the differential foraging activity of honeybees in relation to aspects of crop management including sowing time and parental row ratio in the hybrid seed production of the first Indian mustard hybrid NRCHB 506. *Apis* spp. (half of which being *Apis dorsata*) was the dominant pollinators in our study. The pollinator abundance was maximum in the forenoon in male line (19.1) and in the afternoon in female line (13.9). Pollen collectors (8.7) outnumbered the nectar collectors (7.2) in male line and the reverse in female line. Pollinator abundance decreased in rows distantly positioned from the male parent row. Air temperature was positively correlated (r = 0.631) and relative humidity negatively correlated (r = –0.736) with honeybee foraging. Warmer weather conditions during flowering period showed higher number of honeybee visiting the hybrid seed production plots. This study would help the seed producers to standardise the planting geometry and date of sowing to promote maximum pollinator abundance at peak flowering period resulting in higher hybrid seed yield.

Key words: Date of sowing, Honeybee activity, Indian mustard, Row ratio, Weather condition

More than 50 per cent of the existing species of plants propagated by seeds are dependent upon insects for adequate pollination (Mohapatra *et al.* 2003). Bees contribute the yield of cross as well as to some extent in self-pollinated crops through pollination. It has been reported that there are more than 25000 described species of bees in the world and it accounts for 65 per cent pollination of various flowering plants (Eckert 1933). Bee can travel several kilometres to forage, but usually foragers concentrate within 0.8 km from the hive when a suitable resource is available. Many studies have shown that a large proportion (up to 80 per cent) of bee flights are less than 1 m in distance, with the majority of pollen being transported by bees less than 5 m (Cresswell 1999, Ramsay *et al.* 1999, Pierre 2001). Occasionally however, bees may travel much further and studies have measured bee flight distances of 1 - 2 km (Eckert 1933), up to a maximum distance of 4 km (Ramsay *et al.* 1999), Thompson *et al.* 1999). The mean distance of pollen dispersal is dependent not only on pollinator behaviour but also plant density as sparse areas of plants receive far fewer pollinator visits (Kunin 1997). Given abundant flowers, such as in cultivated fields, individual honey bee foragers tend to collect nectar and pollen from flowers in the same or immediately adjacent plants. Honeybees differ with respect to collection of pollen, nectar or both. It was reported that 58, 25 and 17 per cent of honeybees foraging on mustard were nectar collectors, pollen collectors or both nectar and pollen collectors, respectively (Atwal 2001). Insect foraging depends on many factors including spatial arrangement of plants, environmental conditions, plant density and availability of pollen (Rieger *et al.* 2002). Bees search a larger region for food during flowering; honeybees forage during daylight and are unlikely to carry pollen grains viable to effect fertilisation beyond 1200 hours (Kraai 1962). The number of flowers visited per minute by any bee species depends upon a number of factors including instinctive foraging behaviour, length of proboscis and floral structure (Free 1993), particularly the corolla depth, type and quantity of floral rewards, density of flowers of particular cultivar of the crop grown and the time of the day. The breeding techniques have altered the value of nectar and pollen rewards, synchronised phenology and changed visual cues to such an extent that pollinators confine their visit to one parent in case of hybrid seed

[1] Scientist (e mail: maityam@gmail.com), Seed Technology Division, Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh 284 003; [2] Principal Scientist (e mail: skchakra_sst@yahoo.com), [3] Technical Assistant (e mail: jadavjb@yahoo.com), Division of Seed Science and Technology

production, thereby reducing the pollination of female line in sunflower (Skinner 1987). In different oilseed crops, peak foraging activity of honeybees synchronised with anthesis (Sihag and Khatkar 1999). Knowledge of favourable planting geometry of male and female parents and other agronomic and weather conditions to promote pollinator activity and movement between both male and female lines are required for maximization of hybrid seed production.

Parental lines of NRCHB 506 had distinct differences with regard to their attractiveness. The male parent MJR1 is taller with larger flowers and more robust and attractive anthers than those of the female parent MJA5. So, it is essential to understand the foraging behaviour of honeybee species in the parental lines of the hybrid in relation to differential planting geometry and weather conditions to develop a hybrid seed production technology in this first Indian mustard hybrid.

MATERIALS AND METHODS

The female (MJA5) and male line (MJR1) seeds of the Indian mustard hybrid NRCHB506 were sown in plots with sufficient soil moisture, in three different dates, i.e. 21 October (D1), 30 October (D2) and 18 November (D3) following a planting ratio of 2:16 (R:A) in 2009. The row length was 5m with spacing between rows and between plants within a row (after thinning) as 50cm and 20cm respectively. The experiment was laid out in an RBD with three replications each with three sets of plots in 2:16 (R:A) planting ratio. In 2010, parental lines were shown in three different planting ratios, i.e. 2:8, 2:12 and 2:16 (R:A) in three replications (three sets in each replication) in the third week of October in 2010. The plots were isolated by at least 100 m from each other in both the years. Weather data were recorded at the specified time simultaneously on all the days when honeybee visit was recorded. Number of bees visited in two minutes period was observed in the morning (10 to12 am) and afternoon (2 to 4 pm) on three randomly selected plants in each row of male and female parents up to middle row of each of the three planting ratios in three dates of sowing. It was observed for three consecutive days in each plot at peak flowering time. Time spent by a bee from the time it lands on an inflorescence until it flies away after foraging was recorded with the help of a stopwatch. These observations were recorded between 10.00-12.00 hours (forenoon denoted as F) and 14.00 -16.00 hours (afternoon denoted as A) for three days in each row in all dates of sowing and planting ratios. Bees with pollen in their corbicula were recorded as pollen gatherer (denoted as P) and bees without pollen load were counted as nectar collector (denoted as N). A randomised block design was used for all the above-mentioned experiments. The obtained data were statistically analysed using analysis of variance (ANOVA) and means were compared and grouped by using the Least Significant Difference test (LSD 0.05) with the SAS 9.1.3 programme (SAS Institute 2004).

Table 1 Distribution of pollinators in hybrid seed production plot of NRCHB506

Species	Proportion (%)	
	Female line	Male line
A. dorsata	25	26
A. mellifera	20	20
A. cerena indica	19	19
A florea	17	18
Others	19	17

Table 2 Relationship(s) between foraging rate and weather parameters during peak flowering period

Parameter	No. of honeybees/2 min/plant
Temp.	0.631^{NS}
RH	-0.736^{*}
Sunshine hr.	0.696^{*}
Wind speed	-0.837^{**}

NS: non significant, *: significant at P = 0.05 and **: significant at P = 0.01)

RESULTS AND DISCUSSION

Distribution of honeybee species in male and female parental lines

Honeybees (*Apis* spp.) were the dominant pollinators and among these, *Apis dorsata* was predominant with 25% and 26% alone in female and male line respectively followed by *A. mellifera*, *A. cerena indica* and *A. florea* (Table 1). The distribution of honeybee species was similar in case of male and female parents.

Relationship between honeybee activity and weather conditions

Honeybee abundance increased with increase in average daily maximum temperature (r =0.631) and with increase in effective sunshine hours (r = 0.696). But relative humidity and wind speed showed negative correlation with honeybee population (r = –0.736 and –0.837, respectively) which resulted in their low yield (Table 2).

Effect of date of sowing on bee foraging rate, mode, time of the day and time spent on inflorescence

Number of honeybee (counted as number of honeybee per two minutes period per plant) increased significantly with delay in sowing time (Fig 1) in both male and female parental lines with the highest value in the third sowing (16.5 and 15.2 for male and female line, respectively). Regarding foraging mode, in female line the number of pollen gatherer and nectar collector increased significantly from D1 (5.2 and 7.2) to D3 (5.9 and 8.2). In male parent, pollen gatherers (8.7) increased significantly (8.5 to 9.0) but not the nectar collectors in later date of sowing (Fig 2). Date of sowing didn't effect bee activity in different foraging time in male parental line but had significant influence on

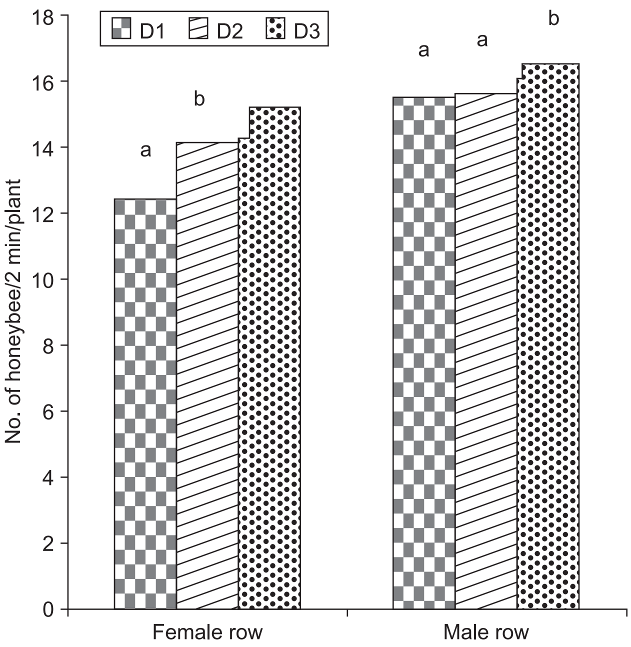

Fig 1 Effect of date of sowing on honeybee foraging (no. of honeybee /2 min/plant) in female and male parent (means following same letter are not significantly different at P = 0.05)

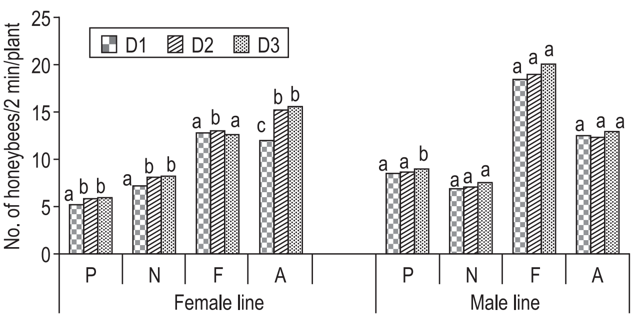

Fig 2 Effect of date of sowing on honeybee activity (no. of honeybees/2 min/plant) with respect to foraging mode and time in female and male line (means following same letter are not significantly different at P = 0.05; P= no. of pollen collecting honeybees/2 min/plant, N= no. of nectar collecting honeybees/2 min/ plant, F= no. of honeybees /2 min/ plant at forenoon and A= no. of honeybees/2 min /plant at afternoon)

the same in female line (Fig 2). Foraging mode in different row position also varied with respect to date of sowing. Proportion of pollen to nectar collector honeybees was higher in D3 in all the row positions (Fig 3). Time spent per inflorescence in the forenoon was highest in D2 (12.25 second) but reduced significantly in D3 (10.1 second). In the afternoon, it varied significantly in all the dates of sowing.

Effect of planting ratio of parental line on bee foraging rate, mode, time of the day and time spent on inflorescence

Planting ratio had also shown a significant effect on honeybee foraging rate. When female to male planting row ratio was increased, the honeybee foraging rate decreased significantly. Highest value, *i.e.* 16.0 in male parental rows

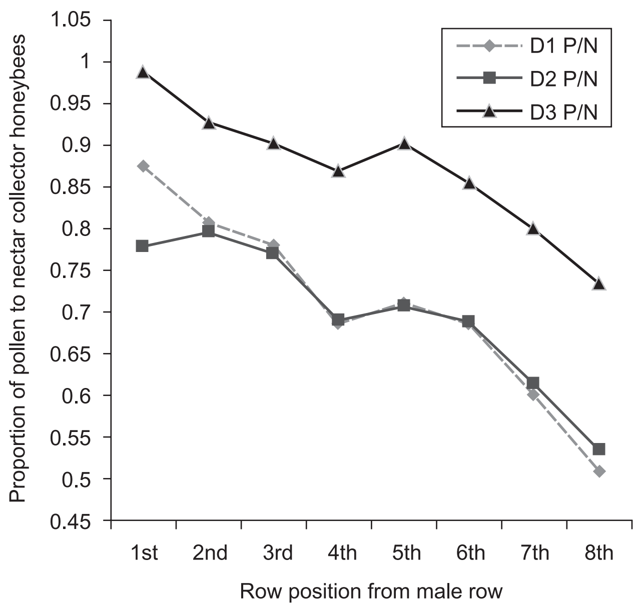

Fig 3 Effect of date of sowing on proportion of pollen to nectar collector honeybee in different row position (means following same letter are not significantly different at P = 0.05; P= no. of pollen collecting honeybees/2 min/plant, N= no. of nectar collecting honeybees/2 min/plant)

and 14.3 in female parental rows was observed in 2:8 (male to female) planting ratio. Higher number of honeybees was recorded to forage in the forenoon in male as well as in female line. Pollen collecting mode decreased significantly in later date of sowing (6.8 in 2:8 to 5.4 in 2:16) in female line but increased in male line (8.5 in 2:8 to 9 in 2:16). Foraging mode in different row position also varied with respect to planting ratio. Proportion of pollen to nectar collector honeybees was higher in 2:8 (R:A) ratio and even in the 4th row it was higher than other planting ratios. The maximum bee abundance was found in the first row adjacent to the male parent row in all the cases. Time spent per inflorescence by a honeybee significantly reduced in wider planting ratio in the forenoon but increased in the afternoon. The honeybee foraging counted in each date of sowing was compared with the respective average plant yield (g) (Fig 4). Hybrid seed yield/plant (g) was primarily contributed by

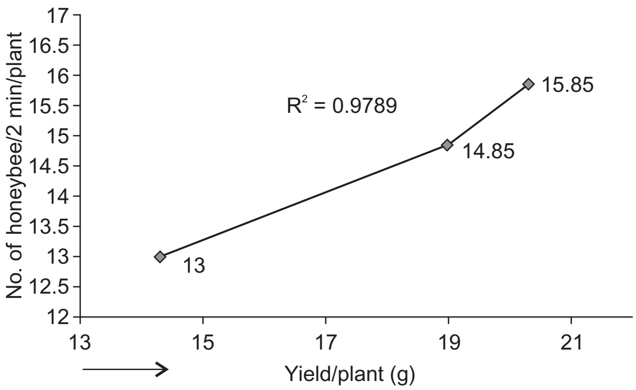

Fig 4 Relationship between honeybee activity and plant yield (g) at different date of sowing

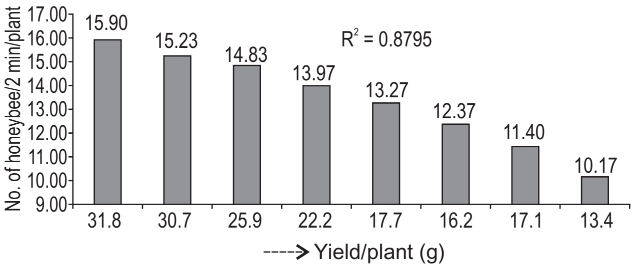

Fig 5 Relationship between honeybee activity and plant yield (g) in different row position

honeybee foraging (R^2= 0.9789). Row wise mean hybrid seed yield per plant (g) plotted against the row wise honeybee activity showed that honeybee foraging had very high effect on hybrid seed yield (R^2= 0.8916) (Fig 5).

Honeybees were the predominant pollinating agents in hybrid seed production plot in both the years. Sinha and Chakrabarti (1980) reported the build of colony after overwintering necessitates more pollen grains to feed the larvae. Studies on annual foraging cycle of *A. dorsata* (Nagaraja and Rajagobal 2000), *A. mellifera* (Nagaraja 2000), *A. cerana indica* (Reddy 1980) and *A. florea* (Nagaraja and Rajagobal 1999) support these findings. Similar findings were reported in sunflower hybrid seed production by Yadav (2002). Studies on bee abundance on both parental lines revealed that honeybees discriminated both the parental lines due to the difference in flower attractiveness and floral rewards. This was in accordance with earlier reports on brussels sprout (Free and Williams 1983) in which the variation between parental lines with regard to height, flower colour and unknown factors was observed. Singh *et al.* (2006) reported that the absence of pollen collectors in female line in hybrid seed production of *Brassica napus*. Confinement of pollinators in one parent resulted in reduced pollination in sunflower (Skinner 1987). Woyke (1989) found that in *Brassica* higher nectar production (1.5 to 3.8 times as much) was the main reason for more bee visit on female parent. Present investigation revealed enhanced foraging of honeybees in male line due to presence of both pollen and nectar. This report is contradictory with previous findings of Mohr and Jay (1988), Singh *et al.* (2006) and Skinner (1987). To compensate this problem bee hives are commonly kept in bee-pollinated crop plot to facilitate pollination and to maximise seed set (Nieuwhof 1963).

Maximum honeybee activity was recorded in the forenoon in male lines as it provides more floral rewards in terms of pollen which is regarded as source of protein. In female line bees visited more in the afternoon when pollen in male line gets exhausted. A diurnal rhythm of honeybee visit is influenced by change in climatic conditions. Bee visits commenced only if the threshold level of temperature and light intensity is surpassed (Sihag 1984). During forenoon, bees receive optimum temperature and light intensity to increase their foraging activity. Besides, peak anthesis takes place during this time. The maximum abundance of all insect pollinators at morning hours is due to the variation in atmospheric conditions and availability of floral rewards from large number of freshly open flowers (Priti and Sihag 1998, Sihag and Khatkar 1999). Singh *et al.* (2000) reported that peak foraging activity of bees on sunflower was at 6.00 hours followed by 16.00 hours and 10.00 hours. This might probably be due to difference in the calorific rewards. Pollen collecting bees are very active in the morning hours when pollen is available in plenty after anthesis. Within few hours as temperature rises nectar is secreted in flowers and the nectar collecting bees become active for rest of the day. The synchronisation of activities of both these components is the prerequisite for the success of mutualistic relationship between plant and pollinators. Free (1993) stated that the metabolic activity of insects increases as the temperature increases and they visit many flowers at that time. In our study, the time spent per flower was more at forenoon due to intensive pollen gathering activity by most of the bees. It is supported by Singh *et al*. (2006) and Selvakumar *et al*. (2007). As the time was more per flower, number of flowers visited per minute was less in the forenoon. Time spent per flower indicates thoroughness of the species in pollination and time spent per plant provides information on relative constancy of various bee species on a plant. This was due to availability of more floral reward up to midday. Pollen gatherers spent 131 seconds and nectar collectors spent 94 seconds per plant in Kale inflorescence (Free and Williams 1983). In this study it spent 10.1 to 13.4 seconds on case basis. Foraging duration and foraging rate varied among different sowing dates and planting ratios. Regarding difference in honeybee activity in different planting ratios, Maity *et al*. (2012a and 2012b) reported that planting ratio doesn't affect flowering and morphological characters of parental lines in hybrid seed production plot. Therefore, higher honeybee activity in narrower planting ratio may be due to more abundance of pollen parent that provides more floral rewards in the field. Regarding difference in honeybee activity in different dates of sowing, Joshi and Joshi (2010) and several other authors (Al-Qarni 2006, Blazyte-Cereskiene *et al.* 2010, Human *et al.* 2006, DeGrandi-Hoffman *et al.* 2000) have reported that honeybee activity for pollination in different crops is a function of weather parameters. Among the environmental factors, temperature and sunshine hours were positively correlated with honeybee activity while RH and wind velocity were negatively correlated in the present study. Honeybee is homeo-thermal in nature, as the colony can regulate their body temperature (Sihag 1984). However, an individual honeybee is poikilothermic and it cannot regulate its body temperature (Michener 1974). The individual bee strictly follows the directions of this factor. Difficulty in locating and communicating food sources under low light intensity limits foraging activity. That is why foraging activity has shown negative correlation with sunshine hours. Under favourable weather condition, the intensity of light does not affect bee activity much more. But when weather is cold and there is a food shortage in a colony, the bees are stimulated by bright light to forage even though the outside temperature is very low. In our study both sunshine hour and temperature increased on later date of sowing influencing the bee activity in positive direction. Thus environmental factors had great influence on the foraging behaviour of honeybees, indicating the need of optimising favourable pollination dynamics to maximise hybrid seed yield.

ACKNOWLEDGEMENTS

We are thankful to Dr K H Singh, Directorate of Rapeseed-Mustard, Bharatpur, Rajastan, India who provided the seeds of parental line.

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