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Effect of Different Nest Box Materials on the Mating of European Bumblebee, *Bombus terrestris* (Hymenoptera: Apidae) under Controlled Environmental Conditions

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Abstract.-We examined the possible effect of different box materials in relation to time interval on the mating of European bumblebee, *Bombus terrestris* under controlled environmental conditions. The lowest rate of mating was found in wooden box with mean of 49% pairs and the highest mating tendency was found in polycarbonate box. Further in relation to time interval, during the first minutes of introduction of queens and males in mating cage, significant difference between all three boxes showed the highest mating rate within first minutes in polycarbonate box. Similarly, during the five minutes of time interval from introduction, mating tendency was high in polycarbonate box which was 1-2 fold higher from iron mesh box and wooden box. During the time elapse of fifteen minutes of introduction of queens and males in the mating cage, there existed no significant difference between the polycarbonate box and iron mesh box but significantly different in wooden box. Such important abiotic affect of mating environment suggests the possibilities for higher mating tendency needed for their commercial breeding programs.

Key words: *Bombus terrestris*, mating requirement, box material, time interval.

INTRODUCTION

Bumblebees are imperative pollinators of a variety of greenhouse crops mainly effective in pollinating for the solanaceous family commercial plants like eggplant and tomato (Buchmann and Hurley, 1978; Free, 1993). Their effective crop pollination and flower visitation inside enclosed farming systems has given the growers the chance to reduce the work expenses of pollination and assure high quality yield (Iwasaki, 1995). Bumblebee and honeybees keep the record of suitable host plants for food reserves and best pollinator with their rapid learning abilities (Ali *et al*., 2014). Bumblebees are the most commonly used pollinators for the pollination of mostly greenhouse crops. Five different bumblebee species *viz.*, *Bombus terrestris* L., *B. lucorum*, *B. occidentalis*, *B. ignitus and B. impatiens* are reared commercially to meet the needs of farmers for crop pollination with *B. terrestris* being the most commonly used species. With increase in area under enclosed farming of vegetables and fruits, their demand has increased with time and more than a million colonies of the bumblebees per year are used by the farmers to meet their needs (Velthuis and Doorn, 2006). Indigenous bumblebee species, *B. haemorrhoidalis* Smith has been considered important to rear and use for greenhouse pollination (Umer *et al*., 2014)

Bumblebee produces large colonies and adapts quite well to artificial environment. Even though the production techniques have been developed since 1987 of its commercialization, some problems of increasing the quantity and quality of artificially reared colonies in commercial rearing is still felt (Hughes, 1996). There exist variation in the number of males, workers and daughter queens produced (Beekman and van Stratum, 2000). Queens are the only caste to overwinter (enter diapause), during the last stage of colony sexuals are produced and mating is done after mating queens went to diapause and remaining males and workers die in the colony. In early spring queens that overwintered leave their hibernation sites. The queen builds up a mass of pollen and lays her first batch eggs into the pollen mass after searching appropriate place to found a colony. Once

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the workers of the first brood have emerged they take over the foraging duties of the queen, who from now on spends her time mostly on the laying of eggs. In the late summer, many males and new queens are produced and only mated queens hibernate and emerge in spring (Heinrich, 2004; Duchateau and Velthusis, 1988).

An important stage in the life of bumblebees is mating. *B. terrestris* males consist of patrolling performance during which secretions from the cephalic labial gland are deposited on marking spots (Schmid-Hempel and Schmid-Hempel, 2000). Males of bumblebee released special pheromones during flight by scent-marking plants and they try to copulate with young queens encountered on their flight-path (Svensson, 1980; Williams, 1991). The special type of pheromones which secreted by the male from the cephalic labial gland has been identified as farnesol. The young unmated queens also secrete sex pheromones from their mandibular glands to attract the males (van Honk *et al*., 1978; Bergstrom, 1981). Djegham *et al*. (1994) produced a precopulatory ethogram for *B. terrestris* in a cage where no nuptial route could be established.

Male–male rivalry over access to females has led to the evolution of various adaptations to make the most of male mating success and paternity among offspring. *e.g.*, during mating males may transfer not only sperm, but also chemical substances into the female's sexual tract, which increase the likelihood of paternity (male mating success) (Price *et al*., 1999; Gonzalez *et al*., 1999; Soller *et al*., 1997). Such chemical compounds are produced in the male accessory gland of insects (Gillott, 1996) known to induce behavioral and physiological changes in the mated female insects (Wolfner, 1997; Yi and Gillott, 1999).

Mating success in social insects is especially interesting. In particular, whether one or several males succeed in mating with a single queen affects the average relatedness among workers in the colony. This leads to variation in a number of key parameters of sociality such as the level of cooperation (Keller and Reeve, 1994) or sex allocation to offspring (Boomsma and Grafen, 1990). For the social Hymenoptera information on queen mating behavior is available but male reproductive skews, *i.e.*, sperm bias are poorly investigated including the function and biology of the male accessory gland. Given the observed variation in female mating frequency and sperm bias (Boomsma and Ratnieks, 1996), more information is needed about the function and the biochemistry of these compounds in social insects. This could help in understanding recently discussed topics such as the observed differences in queen mating frequencies among females and among taxa.

Queens of *B. terrestris* appear to be singly mated in Central Europe (Schmid-Hempel and Schmid-Hempel, 2000). During the personal observation, they often refused to re-mate in flight cages although queens would benefit from multiple mating by higher fitness and lower parasite loads (Baer and Schmid-Hempel, 1999). Therefore, queens do not take advantage of the benefits of multiple mating. Different abiotic conditions have their considerable contribution in mating success of bumblebees including specific intensity light frequency, temperature, relative humidity, photoperiod, food and biotic conditions including age and size or weight of the males and virgin queens (Leather *et al*., 1993; Germ and Kral, 1995; Kwon *et al*., 2006; Yoon *et al*., 2007; Amin *et al*., 2010, 2012). In this study we have investigated the effect of different box materials on the mating of the European bumblebee.

MATERIALS AND METHODS

Experimental insects

Present work was conducted to study the effect of box material on mating of the European bumblebee, *Bombus terrestris* L. under standard laboratory conditions in Non-Apis Bee Laboratory, Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi during 2013-2014. Experimental daughter queens and males of *B. terrestris* were obtained from bumblebee hives imported from Koppert Biological Systems, Netherlands and reared on fresh and frozen honeybee collected pollen with commercial grade sucrose sugar at 50% concentration. These bee hives were assigned numbers for identification and collection of daughter queens and males kept in different boxes with nectar and pollens based on age and mother colony number to distinguish. Daughter

queens were mated with the males of same species belonging to different bumblebee hives to avoid inbreeding problems. After mating in special mating cages, daughter queens were feed for about a week period and kept for diapause in refrigerator at 2.5 degree centigrade for 2.5 months period after CO_2 exposure (Tasei and Aupinel, 2008). Hibernated queens were, later, removed from low temperature environment and kept in a cage for CO_2 nacrosis (Velthuis and Doorn, 2006).

Climatic conditions in rearing room

The basic colony-rearing technique was followed as described by (Yoon and Kim, 2002). The experimental groups were reared in three sizes of plastic made boxes with clean hard card paper for nest initiation. Colony initiation or small (17 × 12 × 10 cm), colony foundation or medium (22.0 × 17.0 × 10.0 cm) and colony maturation or large box (25.0 × 25.0 × 25.0 cm) were provided as per need of the colony stage. In all types of nest boxes, small holes were equipped at front wall of the chamber and at both sides of the walls. To minimize the risk of fungus inside, rearing boxes were checked and cleaned every day with a piece of cotton soaked with 75% ethyl alcohol in fume hood to remove the maximum of alcohol to evaporate before reuse. Glass plates were used as lids and covered with black sheet. The climatic conditions were kept at 25±2°C and 60±10% relative humidity (Duchateau and Velthuis, 1988).

To examine the effect of box material on the mating efficiency of bumblebee, we used three types of box *i.e.*, made of wooden material, iron mesh and polycarbonate box with same internal size of $0.5\times0.7\times1m^3$ each. At the time of climax when queen of colony switch to produce males and daughter queens, these all newly emerged males and queens were collected from the colonies and placed in separate boxes with emergence date and assigned a specific number to each box to prevent inbreeding problem. These newly emerged males and queens were fed with 50% sugar solution and fresh pollen collected from honeybee hive and these males and queens and kept at standard laboratory conditions (Duchateau and Velthuis, 1988).

When the age of queens reached at seven days and males at ten days from the ones kept from different colonies with proper marking, they were then used for the experimentation of their mating tendency. In each box, 1:2 ratios of males to queens *i.e.*, 15 queens and 30 males were used to mate and there were fifteen replications in each box. After the introduction of males and queens in the mating cage, data were recorded in three intervals for total number of pairs within first one minute, total number of pairs within first five minutes and total number of pairs within fifteen minutes. Overall mating percentage was also recorded in tested three mating cage types.

Data analysis

Means of different mating parameters were subjected to statistical methods using means ± SD and compared with ANOVA at 5% probability for comparison of percentage values. Chi-squares analysis was performed using SPSS software (NorusICE SPSS Inc. 2006).

RESULTS

A total of 675 daughter queens and 1350 males of *B. terrestris* were used in this experiment to observe the mating tendency in relation to three different type of mating box made with different materials.

Single mating rate within one minute of introduction

Latency (time between introduction of queens and males into mating cage until their copulation initiation) showed significant variation on mating rate of bumblebees during first minute in the mating cages. Highest pairing occurred in polycarbonate box (3.66±1.95) followed by iron mesh box and wooden box (1.86±1.18, 0.46±0.74), respectively. Box material showed significant affect on the mating tendency during first minutes of released ($df= 2, 44$ $F= 20.1$, $P< 0.05$) (Table I). Copulation rate was 24% in polycarbonate box followed by 12% and 3% in iron mesh box and wooden box within one minute of sexuals introduction (Fig. 1).

Single mating rate within five minutes of introduction

After five minutes of introduction of sexuals in mating cages, the highest number of pairs were

Table I.- Effect of boxes material on mating of *Bombus terrestris* with different interval of time period.

Mating boxes	Copulation within 1 minute (pairs in number)[a]	Copulation within 5 minutes (pairs in number)[a]	Copulation within in 15 minutes (pairs in number)[a]	Total mating % (pairs)[a]
Wooden box	0.47±0.74 c	1.46±1.25 c	3.73±1.57 a	49.3±9.01 c
Iron mesh box	1.9±1.18 b	2.66±1.17 b	3.33±1.29 a	61.7±8.1 b
Polycarbonate box	3.66±1.95 a	4.33±1.54 a	2.33±0.81 b	75.1±10.5 a

[a] Values are presented in mean ± Standard deviation
Mean followed by different letters in the column are significantly different at P<0.05 by Least Significant Difference Test

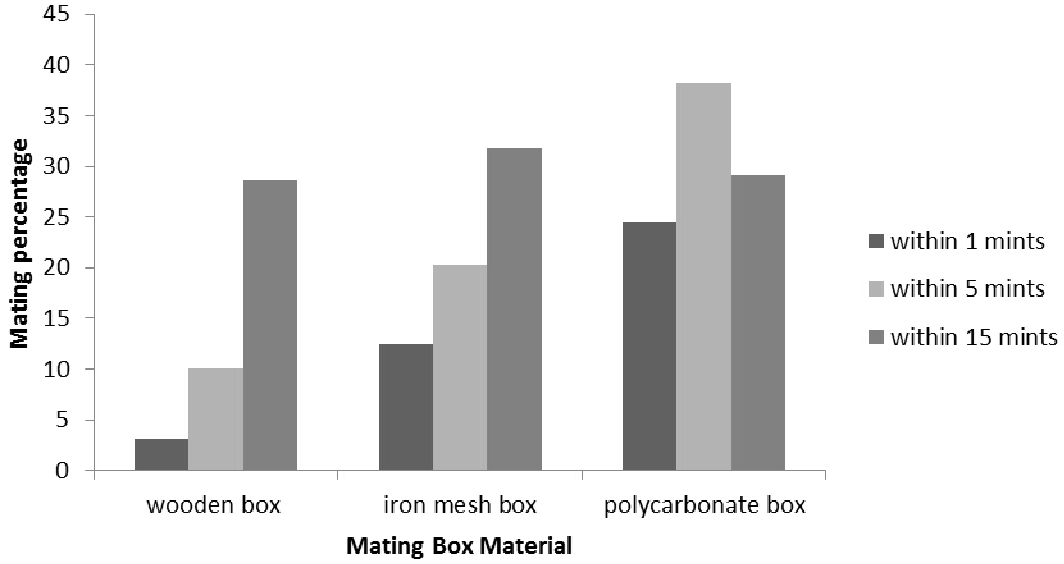

Fig. 1. Relationship between time interval and mating boxes material on mating efficiency of *Bombus terrestris.*

collected from polycarbonate box (4.33±1.54) followed by 2.66±1.17 in iron mesh box. The percentage of copulation observed in wooden box was 1.46±1.25 (F= 17.6, P<0.05) (Table I). Copulation rate was 38% in polycarbonate box followed by 20% and 10% in iron mesh box and wooden box, respectively within five minutes of sexual introduction (Fig. 1).

Mating rate within fifteen minutes of introduction

There appeared non-significant difference for bumblebee mating rate in wooden box (3.73±1.57) and iron mesh box (3.33±1.29) during the fifteen minutes of time interval from the introduction of queens and males, however, both were significant than polycarbonate (2.33±0.81) (F= 4.99, P< 0.05) (Table I). Copulation rate was 29% in polycarbonate box followed by 32% and 28.4% in iron mesh box and wooden box, respectively within fifteen minutes of sexuals introduction (Fig. 1).

Box material showed significant affect on the mating tendency (df= 2, 44, F= 28.9, P< 0.05). Some males and daughter queens started mating and number of mating tendency differed in different treatments (Table I). Mating percentage was the highest in polycarbonate box (75.1±10.5%) followed by iron mesh box and wooden box (61.7±8.1%, 49.3±9.01%), respectively. Out of 225 queens released in, polycarbonate box 169 queens mated whereas 139 and 111 queen mated with males in iron mesh box and wooden box, respectively. Overall result showed that polycarbonate plastic box

is the most suitable for mating during three times observed followed by iron mesh box and then wooden box.

DISCUSSION

Different box materials and time elapse of males and queen introduction showed a strong effect on the mating success of *B. terrestris*. The highest mating success was found in the polycarbonate box. This might be due to transparent character of this mating cage making light availability significantly more. In this mating cage, 1000 to 2000 lux light intensity was observed which is considered most effective for mating of *B. terrestris* inside controlled mating (Yoon *et al*., 2007). Natural mating of bumblebees has been observed during day light and photoperiod had shown pronounced affect on different behaviors of bumblebees especially the mating tendency and success rate (Leather *et al*., 1993; Kwon *et al*., 2006). Light assert the sexual maturity and pheromone production and may change in behavior by changing neurotransmitter concentration in brains (Germ and Kral, 1995). Clear and transparent surface of polycarbonate mating cage might have influence to resist negligible for light to penetrate and actively affect the mating rate inside.

Other environmental factors like temperature, relative humidity, food and enclosed environment has impact on different biological life process including mating in bees (Kwon *et al*., 2006). Copulation rate in bumblebees is also influenced and optimum temperature observed previously was 23^{o}C proving to be the most favorable for this important life continuing process in the solitary bee, *B. terrestris* (Amin *et al*., 2010). We maintained temperature of the mating room around 20-25^{o}C which was almost same inside the polycarbonate mating cage providing the best temperature environment. Wooden box needed more time to attain the desired temperature which desired the upper surface of the wooden mating cage to remain open of covered with polycarbonate sheet. There might have been some inclusive impact of that surface. We observed overall maximum copulation rate in iron mesh cage where the temperature was almost the same of that in the mating room. This, however, was close to that observed in polycarbonate mating cage.

Material of the mating cage, thus influenced this copulation process and directly proportional on mating latency (time elapsed between the introduction of queens and males in mating cage until initiation of copulation). During the first and five minutes of introduction of males and queens to mate, mating rate was the highest in polycarbonate box and lowest in wooden box. Major factors affecting time latency might include the light intensity and temperature. In natural bumblebee life systems, males patrol and scent the routes with their pheromones to attract females to visit these routes and get copulated by these males (Schmid-Hempel and Schmid-Hempel, 2000). The activity of males for patrolling and marking is highly affected by the temperature and optimum light. Low light intensity in wooden and iron mesh mating cages might have impact on the copulation rate. Copulation time period was more in these cages and queens required longer time to detect each other (Bourke, 1997; Estoup *et al*., 1995). Heavy males were quick in selection of virgin queens to mate which lasted for less time than other males to increase the mating success rate, however, their age have no significant affect (Amin *et al*., 2010, 2012). Combination of different abiotic conditions in mating success of bumblebees include specific intensity light frequency, temperature, relative humidity, photoperiod, food and biotic conditions of age and size or weight of the males and virgin queens have their influence, however, the environment for their mating also highlights the material of the cage used for their mating success and tendency (Leather *et al*., 1993; Germ and Kral, 1995; Kwon *et al*., 2006; Yoon *et al*., 2007; Amin *et al*., 2010, 2012).

In conclusion, mating or copulation rate of bumblebees in relation to mating cage material and time interval are of practical relevance wishing to induce mating in bumblebees for commercial breeding systems of these bees. Polycarbonate box material proved the most suitable for bumblebee mating in enclosed laboratory rearing or breeding programs to achieve maximum copulation and enhance their mated queen to initiate more hives for next cycle. We believe such studies will be helpful regarding mating behavior for effective bumblebee

rearing programs and contribute more for enhancing their role in crop pollination of enclosed farming systems with better qualitative and quantitative production and good economic returns.

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